

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
13 December 2001 (13.12.2001)

PCT

(10) International Publication Number
WO 01/94600 A2

(51) International Patent Classification⁷: C12N 15/63

(21) International Application Number: PCT/US01/18343

(22) International Filing Date: 6 June 2001 (06.06.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/209,549 6 June 2000 (06.06.2000) US

(71) Applicant: GENELABS TECHNOLOGIES, INC.
[US/US]; 505 Penobscot Drive, Redwood City, CA 94062
(US).

(72) Inventors: KIM, Jungsuh, P.; 1844 Guinda Street, Palo Alto, CA 94303 (US). STARR, Douglas, B.; 1197 Bonita Avenue, Mountain View, CA 94040 (US). TAM, Albert, W.; 1871 10th Avenue, San Francisco, CA 94122 (US). LAURANCE, Megan, E.; 1020 Shotwell Street, San Francisco, CA 94110 (US). MICHELOTTI, Emil, F.; 1181 Marlin Avenue, Foster City, CA 94404 (US). VELLIGAN, Mark, D.; P.O. Box 370532, Montara, CA 94037 (US). LATOUR, Derek, R.; 1945 East Avenue, Hayward, CA 94541 (US). THOMAS, Rita, L.; 1050 High Road, Woodside, CA 94062 (US). KONGPACHITH, Ana; 4469 Alvarado Blvd., Union City, CA 94587 (US). SHEPPARD,

Liana, T.; 34 Cadiz Circle, Redwood City, CA 94065 (US). LIM, Moon, Young; 10 Lido Circle, Redwood City, CA 94065 (US). BRUICE, Thomas, W.; 6880 Shearwaters Drive, Carlsbad, CA 92009 (US).

(74) Agents: JUDGE, Linda, R. et al.; Iota Pi Law Group, P.O. Box 60850, Palo Alto, CA 94306-0850 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/94600 A2

(54) Title: PROMOTERS FOR REGULATED GENE EXPRESSION

(57) Abstract: This invention provides nucleic acid sequences, vectors and host cells comprising regulatory regions associated with various promoters including a cyclin D1 promoter, a CD40L promoter, three HBV promoters (core, pre-S1 and HBV-X), a vancomycin-resistant enterococci (VRE) promoter, an androgen receptor promoter, a Her2 promoter, and a β -lactamase promoter. The invention further provides methods of regulating gene expression comprising the regulatory regions of such promoters.

1/11/2002

THIS PAGE BLANK (USPTO)

Sent out 8 Jan 2002

PROMOTERS FOR REGULATED GENE EXPRESSIONField of the Invention

5 The present invention relates to regulatory sequences within various promoters, and to heterologous nucleic acid constructs, vectors and transformation methods employing such sequences. The invention further relates to modified promoters and their use in regulated gene expression.

References

- 10 Albanese, et al., *Journal of Biological Chemistry* 270, 23589-23597, 1995.
Allen et al., *Science* 259, 990-3, 1993.
Arber et al., *Cancer Research* 57, 1569-1574, 1997.
Arthur M et al., *J. Bacteriol.* 174(8):2582-2591, 1992.
Aruffo et al., *Cell* 72, 291-300, 1993.
- 15 Beier et al., *Proceedings of the National Academy of Sciences (USA)* 96, 1433-1438, 1999.
Biancone et al., *International Journal of Molecular Medicine*, 3:343-53, 1999.
Boyce, et al., *J Clin Microbiol* 32, 1148-53, 1994.
Buckley, *Oncogene* 8, 2127-2133, 1993.
- 20 Buhlmann and Noelle, *Journal of Clinical Immunology*, 16:83-9, 1996.
CDC. MMWR 42:597-9, 1993.
DiSanto et al., *Nature*, 361, 541-3, 1993.
Foy et al., *Annu Rev Immunol.* 14:591-617, 1996.
Gansauge et al., *Cancer Research* 57, 1634-1637, 1997.
- 25 Gauchat et al., *FEBS Letts.* 315, 259-66, 1993.
Graf et al., *Eur. J. Immunol.*, 22, 3191-4, 1992.
Grewal and Flavell, *Immunol Rev* 153:85-106, 1996.
Haldimann, et al., *J Bacteriol.* 179(18):5903-13, 1997.
Hall et al., *Advances in Cancer Research* 68, 67-108, 1996.
- 30 Hancock et al., *Nature Medicine*, 4, 1392-6, 1998.
Handwerger, et al., *Clin Infect Dis* 16, 750-5, 1993.
Herber et al., *Oncogene* 9, 1295-1304, 1994a, 1994b.
Hinz et al., *Molecular and Cellular Biology* 19, 2690-2698, 1999.
Hollenbaugh et al., *EMBO J.*, 11, 4313-21, 1992.
- 35 Holman TR, et al., *Biochemistry* 33(15):4625-31, 1994).
Jiang et al., *Oncogene* 8, 3447-3457, 1993.

- Kay et al., *PNAS*, 94:4686-91, 1997.
- Kirk et al., *Science*, 94:8789-94, 1997.
- Korrmann et al., *Clin. Invest.* 101, 344-352, 1998.
- Korthauer et al., *Nature*, 361, 539-41, 1993.
- 5 Lander et al., *Antimicrob. Agents and Chemo.* 41:1715-1720, 1997.
- Larsen et al., *Nature*, 381:434-8, 1996.
- Lee et al., *Journal of Biological Chemistry* 274, 7341-7350, 1999.
- MA et al., *Proc. Nat. Acad. Sci.* 84:1005-1009, 1987.
- Marshall CG et al., *Biochemistry* 38(26):8485-91, 1999.
- 10 Matsumura et al., *The EMBO Journal* 18, 1367-1377, 1999.
- Moellering RC Jr., *Clin Infect Dis* 14, 1173-8, 1992.
- Motokura & Arnold, *Genes Chromosomes Cancer*, 7:89-95, 1993.
- Murray BE, *Clin Microbiol Rev.* 3, 46-65, 1990.
- Niimi et al., *J. Immunol.*, 161, 5331-7, 1998.
- 15 Ochs et al., *Res Immunol* 145(3):210-5; discussion 244-9, 1994.
- Quelle et al., *Genes and Development* 7, 1559-1571, 1993.
- Philipp et al., *Molecular and Cellular Biology* 14, 4032-4043, 1994.
- Resnitzky et al., *Molecular and Cellular Biology* 14, 1669-1679, 1994.
- Robinson, et al., *J Virol* 14:384-391, 1974.
- 20 Sattler et al., *J Virol* 32:226-233, 1979.
- Schaberg DR et al., *Am J Med* 91(Suppl 3B), 72S-75S, 1991.
- Sherr, *Cell* 73, 1059-1065, 1993.
- Sherr, *Science* 274, 1672-1677, 1996.
- Shimadzu et al., *Biochim. Biophys. Acta*, 1260, 67-72, 1995.
- 25 Shtutman et al., *Proceedings of the National Academy of Sciences (USA)* 96, 5522-5527, 1999.
- Silva JC, et al., *Proc Natl Acad Sci (USA)*, 95(20):11951-6, 1998.
- Spriggs et al., *J. Exp. Med.*, 176:1543-50, 1992.
- Stoll VS et al., *Protein Sci* 7 (5):1147-55, May 1998.
- 30 Sudo et al., *Microbiol. Immunol.* 40(2) 153-159, 1996
- Summers et al., *Proc. Nat. Acad. Sci. (USA)* 72:4597-4601, 1975
- Tetsu et al., *Nature* 398, 422-426, 1999.
- Walsh C, *Science*, 284(5413):442-3, 1999.
- Wang et al., *Nature* 369, 669-671, 1994.
- 35 Watanabe et al., *Journal of Biological Chemistry* 271, 22570-22577, 1996.

Watanabe *et al.*, *Molecular and Cellular Biology* 18, 3212-3222, 1998.

Withers *et al.*, *Mol Cell Biol.* 11(10):4846-53, 1991.

Xiong *et al.*, *Cell* 17;65(4):691-9, 1991.

Yan *et al.*, *Journal of Biological Chemistry* 272, 33181-33190, 1997.

5 Yang *et al.*, *Journal of Virology*, 70, 6370-7, 1996.

Zhang *et al.*, *Cancer Research* 57, 169-175, 1997.

Zhou *et al.*, *Oncogene* 11, 571-580, 1995.

Background of the Invention

10 Gene expression in prokaryotes and eukaryotes is a highly regulated process. Inappropriate expression (over-expression or under-expression) of "normal" or "healthy" genes is associated with many diseases and disease processes. Similarly, expression of mutated genes is also associated with many diseases. Controlling the expression of these genes is one of the ways through which diseases can be treated.

15 All genes contain transcriptional regulatory sequences upstream and downstream from the transcription start site. Transcription factors recognize and bind to transcriptional regulatory sequences and control the production of message transcribed from the gene. Transcriptional regulatory nucleic acid sequences involved in the regulation of gene expression include promoters, enhancers, and regulatory sequences to which
20 transcription factors or transcriptional regulatory proteins bind, which are required for initiation of transcription. Although transcriptional regulatory sequences are most frequently found just upstream of the transcription start site, they can also be found much further upstream, or on the 3' of the gene, or within the introns and exons that make up a gene.

25 A promoter is a region in a DNA sequence generally 1 to about 100 or 200 basepairs upstream of the transcription start site of a gene and typically contains or is adjacent to one or more transcription factor binding sites. An enhancer is a region in a DNA sequence that generally functions to increase transcription of a gene under its control. Enhancers are found upstream and/or downstream from the transcription start
30 site. Enhancers can be located hundreds or even thousands of basepairs away from the transcription start site. Transcription factors bind to promoters and enhancers to regulate transcription.

The sequences of numerous transcriptional regulatory sequences are known in the art, some of which can be found in the "Eukaryotic Promoter Database" developed
35 and maintained by members of the Bioinformatics Group of the ISREC (Swiss Institute

for Experimental Cancer Research), which is available on the Internet. However, absent a thorough analysis of the function of particular sequences found within a given promoter or enhancers, it is impossible to determine whether the particular sequences are important in regulating gene transcription. Once transcriptional regulatory sequences have been identified, they may be utilized to regulate expression of the endogenous genes and may be incorporated into heterologous nucleic acid constructs for use in regulated expression of transgenes. Accordingly, it is of interest to identify and characterize the transcriptional regulatory regions of genes. Of particular interest are the regulatory regions of genes associated with various disease conditions, examples of which are described below.

Mammalian cyclin D1 (CCND1, also named PRAD1 or BCL1) has applications to a number of cancers including but not limited to breast cancers, colon cancers and pancreatic cancers, and plays a critical role in regulating the G₁/S checkpoint of the cell cycle of normal mature animal cells. (See Sherr, 1996)

CD40L ligand (CD40L) (also referred to as gp39, CD154, TRAP or T-BAM) plays a critical role in T cell dependent humoral immune responses by interacting with CD40, which provides a signal needed for T cell activation and recognition of antigen-MHC complexes by the T cell receptor.

Viral induced Hepatitis B (HBV) in humans is estimated to have infected 300 million people worldwide, with a small but significant number of infected individuals developing severe pathologic consequences, including chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma. HBV-specific promoters involved in viral replication are therefore relevant to both therapy of HBV disease and regulated gene expression which is specific to liver cells.

The vancomycin resistance enzyme VanH has been associated with the recently observed increase in the incidence of infection and colonization with vancomycin-resistant enterococci (VRE). Therefore, regulated expression of VanH is relevant to treatment of VRE.

Prostate cancer is the most frequently diagnosed cancer in males in the United States. Current treatments for metastatic prostate cancer involve targeting the androgen receptor (AR) using surgical or chemical means. Regulated expression of the androgen receptor is relevant to treatment of prostate cancer.

Her2 (human epidermal growth factor receptor2; c-erbB2, neu) is a tyrosine kinase growth factor receptor which is overexpressed by breast cancer cells, ovarian

cancer cells and a variety of other cancer cells. Accordingly, regulated expression of Her-2 is relevant to modulating such overexpression.

5 The β -lactamase gene confers ampicillin resistance to *E. coli*. Accordingly, regulated expression of β -lactamase is relevant to modification of such antibiotic resistance.

The present invention provides the sequences of the transcriptional regulatory regions of genes associated with various disease conditions together with a functional characterization of such sequences.

10 Summary of the Invention

The invention is directed to characterization of endogenous regulatory sites in the regulatory region of native gene promoters and their use in regulated gene expression.

15 In one aspect, the invention provides isolated nucleic acid sequences comprising the regulatory region of a cyclin D1 promoter, characterized by the ability to regulate expression of a gene operably linked to a cyclin D1 promoter which includes the regulatory sequence. Exemplary sequences are presented as SEQ ID NO.:5, SEQ ID NO.:6 and SEQ ID NO.:8.

20 In another aspect, the invention provides isolated nucleic acid sequences comprising the regulatory region of a CD40L promoter, characterized by the ability to regulate expression of a gene operably linked to a CD40L promoter which includes the regulatory sequence. Exemplary sequences are presented as SEQ ID NO.:12, SEQ ID NO.:13, SEQ ID NO.:14 and SEQ ID NO.:15.

25 In a further aspect, the invention provides isolated nucleic acid sequences comprising the regulatory region of an HBV promoter, characterized by the ability to regulate expression of a gene operably linked to an HBV core, preS1 or X promoter which includes the regulatory sequence. Exemplary sequences are presented as SEQ ID NO.:20 and SEQ ID NO.:21 (core promoter); SEQ ID NO.:23 or SEQ ID NO.:24 (preS1 promoter); and SEQ ID NO.:26, SEQ ID NO.:27 and SEQ ID NO.:28 (HBV X promoter).

30 The invention also provides isolated nucleic acid sequences comprising the regulatory region of a vancomycin-resistant enterococci (VRE) promoter, characterized by the ability to regulate expression of a gene operably linked to a VRE promoter which includes the regulatory sequence. Exemplary sequences are presented as SEQ ID NO.:32, SEQ ID NO.:33 and SEQ ID NO.:34.

35 The invention further provides isolated nucleic acid sequences comprising the regulatory region of an androgen receptor (AR) promoter, characterized by the ability to

regulate expression of a gene operably linked to a AR promoter which includes the regulatory sequence. Exemplary sequences are presented as SEQ ID NO.:64, SEQ ID NO.:65 and SEQ ID NO.:66.

5 In another aspect, the invention provides isolated nucleic acid sequences comprising the regulatory region of a HER2 promoter, characterized by the ability to regulate expression of a gene operably linked to a HER2 promoter which includes the regulatory sequence. Exemplary sequences are presented as SEQ ID NO.:70, SEQ ID NO.:71 and SEQ ID NO.:72.

10 The invention further provides isolated nucleic acid sequences comprising the regulatory region of an androgen receptor beta lactamase (Bla) promoter, characterized by the ability to regulate expression of a gene operably linked to a Bla promoter which includes the regulatory sequence. Exemplary sequences are presented as SEQ ID NO.:77 or SEQ ID NO.:78.

15 In a related aspect the invention provides a vector comprising a promoter regulatory nucleic acid sequence for any one of: a cyclin D1 promoter, a CD40L promoter, three HBV promoters (core, pre-S1 and HBV-X), a vancomycin-resistant enterococci (VRE) promoter, an androgen receptor promoter, a Her2 promoter, and a β -lactamase promoter, as described above.

20 The vector may be an expression vector which includes the promoter regulatory sequence operably linked to a promoter and control sequences recognized by a host cell transformed with the vector, and a transgene encoding a gene product, e.g., a reporter gene.

25 A host cell comprising such a vector, e.g., a prokaryotic cell, a eukaryotic cell, or a mammalian cell is also provided by the invention. A host cell transformed with such a vector may be used in a method for regulating expression of a transgene and detecting the expression thereof, e.g., by exposing the cell to a cellular factor or a DNA binding compound which interacts with the promoter regulatory sequence.

Brief Description of the Figures

30 Figure 1A presents the sequence of the HBV core promoter.

Figure 1B presents the sequence of the HBV pre-S1 promoter region with the sequences of various DNA response elements (HNF1, HNF3, Sp1 and TBP) indicated as underlined with sequence locations indicated in the figure.

35 Figure 2 depicts the results of a hybridization stabilization assay (HSA) with various HBV preS1 promoter constructs indicating the binding preference of a test

compound, the netropsin dimer, 21x, for the HNF3-wt, TBP-wt, TBP-mut, HNF-1-wt, HNF1-m and HNF1-21x sequences, indicated in the figure.

Figure 3 presents the sequence of the HBV X promoter region with the sequences of various DNA response elements (NF1, 2c, EF-C, NF-1 and X-PBP) indicated as underlined in the figure.

Figure 4 presents the sequence of the wild type cyclin D1 promoter from -1745 to +155, which corresponds to nucleotides 316 to 2161 of GenBank Accession No. L09054.

Figures 5A to C present the sequence of the full-length human CD40L sequence numbered from nucleotide 1 to 2395, wherein nucleotides 10 to 1919 correspond to the human CD40L promoter sequence identified as -1860 to +49.

Figure 6 presents the sequence of the wild type vanH promoter.

Figure 7 presents the sequences of vanH promoter mutants M2-M21, wherein each group of 10 nucleotides in the original vanH promoter sequence shown in the figure was replaced with the mutant sequence, e.g., in M2 the CCCGGGGGGC sequence was inserted in place of the wild type TAATTTTITA sequence.

Figures 8A to C present the sequence of the wild type androgen receptor promoter from -6000 to +1100.

Figure 9 presents the sequence of the wild type Her2 promoter.

Detailed Description of the Invention

I. Definitions

As used herein, the term "polynucleotide" refers to a polymeric molecule having a backbone that supports bases capable of hydrogen bonding to typical polynucleotides, where the polymer backbone presents the bases in a manner to permit such hydrogen bonding in a sequence specific fashion between the polymeric molecule and a typical polynucleotide (e.g., single-stranded DNA). Such bases are typically adenosine, guanosine, cytosine, thymidine, uracil and inosine. Polymeric molecules include double and single stranded ribonucleic acids (RNA) and deoxyribonucleic acids (DNA), and may include polymers having backbone modifications such methylphosphonate linkages.

As used herein, a nucleic acid may be double stranded, single stranded, or contain portions of both double stranded or single stranded sequence. The depiction of a single strand also defines the sequence of the other strand and thus also includes the complement of the sequence.

As used herein, the term "recombinant nucleic acid" refers to a nucleic acid, originally formed *in vitro*, in general, by the manipulation of the nucleic acid in a form not normally found in nature.

5 A "heterologous nucleic acid construct" has a sequence portion that is not native to the cell in which it is expressed. Heterologous, with respect to a control sequence/coding sequence combination refers to a control sequence (*i.e.*, promoter or enhancer) and a coding sequence or gene combination, that is not found together in nature, in other words, the promoter does not regulate the expression of the same gene in the heterologous nucleic acid construct and in nature. Generally, heterologous
10 nucleic acid sequences are not endogenous to the cell or part of the genome in which they are present and have been added to the cell, by transfection, microinjection, electroporation, or the like. Such a heterologous nucleic acid construct may also be referred to herein as an "expression cassette".

As used herein, the term "sequence identity" means nucleic acid or amino acid
15 sequence identity between two or more sequences, when aligned using a sequence alignment program. Sequence searches are preferably carried out using the BLASTN program when evaluating the % identity of a given nucleic acid sequence relative to nucleic acid sequences in the GenBank DNA Sequences and other public databases. The BLASTX program is preferred for searching nucleic acid sequences which have been
20 translated in all reading frames against amino acid sequences in the GenBank Protein Sequences and other public databases. Both BLASTN and BLASTX are run using default parameters with an open gap penalty of 11.0, and an extended gap penalty of 1.0, and utilize the BLOSUM-62 matrix. [See, Altschul *et al.*, 1997.]

The term "% homology" is used interchangeably herein with the term "% identity"
25 and refers to the level of identity between two sequences, *i.e.* 70% homology means the same thing as 70% sequence identity as determined by a defined algorithm, and accordingly a homologue of a given sequence has at least about 70%, preferably about 80%, more preferably about 85%, even more preferably about 90% sequence identity over a length of the given sequence.

30 A preferred alignment of selected sequences in order to determine "% identity" between two or more sequences, is performed using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

A nucleic acid sequence is considered to be "selectively hybridizable" to a
35 reference nucleic acid sequence if the two sequences specifically hybridize to one another

under moderate to high stringency hybridization and wash conditions. Exemplary conditions include hybridization conducted as described in the Bio-Rad Labs ZetaProbe manual (Bio-Rad Labs, Hercules, CA). For example, hybridization is conducted in 1mM EDTA, 0.25 M Na₂HPO₄ and 7% SDS at 60° C, followed by washing in 1mM EDTA, 40mM NaPO₄, 5% SDS, and 1mM EDTA, 40 mM NaPO₄, 1% SDS. Hybridization conditions are further recited in Ausubel FM *et al.*, 1993.

As used herein, the term "vector" refers to a nucleic acid construct designed for transfer between different host cells. An "expression vector" refers to a vector that has the ability to incorporate and express heterologous DNA fragments in a foreign cell. Many prokaryotic and eukaryotic expression vectors are commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art.

As used herein, the term "plasmid" refers to a circular double-stranded (ds) DNA construct used as a vector, which forms an extrachromosomal self-replicating genetic element in many bacteria and some eukaryotes.

As used herein, the term "gene" means the segment of DNA involved in producing a polypeptide, which may or may not include regions preceding and following the coding region. For example, 5' untranslated (5' UTR) or "leader" sequences and 3' UTR or "trailer" sequences, as well as intervening sequences (introns) between individual coding segments (exons), may or may not be included in the DNA segment designated as the gene.

As used herein the term "transgene" refers to the portion of a heterologous nucleic acid construct, expression cassette or vector which comprises the coding sequence for a polypeptide, wherein the gene is associated with other components, *i.e.*, a promoter with which it is not normally associated in nature.

As used herein, the term "DNA response element" may be used interchangeably with the term "regulatory promoter sequence" and refers to the DNA binding site or sequence for a transcriptional regulatory protein, which may be the same as, overlapping, or adjacent to, a compound-binding sequence.

As used herein, the terms "compound binding sequence", "compound binding site", "ligand binding sequence", and "ligand binding site" are used interchangeably and refer to the portion of a DNA sequence with which a compound, ligand, or molecule interacts resulting in the modified binding of a transcriptional regulatory protein to its DNA binding site (or DNA response element). In some cases, the compound, ligand, or molecule may also be designated a compound or inducer. The "compound-binding sequence" or equivalent is in the vicinity of the DNA response element for transcriptional

regulatory protein and may be adjacent (*i.e.*, flanking), overlapping, or the same as the DNA binding site for a transcriptional regulatory protein.

As used herein, the term "promoter" refers to a sequence of DNA that functions to direct transcription of a gene that is operably linked thereto. The promoter may or may not include control sequences (also termed "transcriptional and translational regulatory sequences"), involved in expression of a given gene product. In general, transcriptional and translational regulatory sequences include, but are not limited to, the promoter sequence, include the DNA response element for a transcriptional regulatory protein, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. The promoter may be native or non-native to the cell in which it is found.

As used herein, the terms "regulatable promoter", "inducible promoter" and "switchable promoter", are used interchangeably and refer to any promoter the activity of which is affected by a cis or trans acting factor.

A eukaryotic gene control region consists of a promoter plus regulatory DNA sequences (to which transcriptional regulatory proteins bind). As used herein, the term "regulatory promoter sequence" generally refers to a sequence within the control region of a gene and to which transcriptional regulatory proteins bind, resulting in transcriptional activation or repression. Native forms of such regulatory promoter sequences are generally located 5' to the promoter elements of the gene control region.

As used herein, the terms "transcriptional regulatory protein", "transcriptional regulatory factor" and "transcription factor" may be used interchangeably with the term "DNA-binding protein" and refer to a cytoplasmic or nuclear protein that binds a DNA response element and thereby transcriptionally regulates the expression of an associated gene or genes. Transcriptional regulatory proteins generally bind directly to a DNA response element, however in some cases binding to DNA may be indirect by way of binding to another protein which in turn binds to, or is bound to the DNA response element.

As used herein, the term "operably linked" relative to a recombinant DNA construct or vector means a nucleotide component of the recombinant DNA construct or vector is in a functional relationship with another nucleotide component of the recombinant DNA construct or vector. For example, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous,

and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous.

As used herein, the term "expression" refers to the process by which a polypeptide is produced based on the information contained in a given DNA sequence. The process
5 includes both transcription and translation.

As used herein, the term "modulated expression" refers to a change in transcription and translation, which may represent an increase or a decrease in the amount of a given gene product.

A host cell has been "transformed" by exogenous or heterologous DNA when the
10 DNA has been introduced into the cell. Transformation may or may not result in integration (covalent incorporation) into the chromosomal DNA of the cell. For example, in eukaryotic cells such as yeast and mammalian cells, the transfected DNA may be maintained on an episomal element such as a plasmid.

As used herein, the terms "stably transformed", "stably transfected" and
15 "transgenic" refer to cells that have a non-native (heterologous) nucleic acid sequence integrated into the genome. Stable transformation is demonstrated by the establishment of cell lines or clones comprised of a population of daughter cells containing the transfecting DNA.

In some cases, "transformation" is not stable, *i.e.*, it is transient. In the case of
20 transient transformation, the exogenous or heterologous DNA is expressed, however, the introduced sequence is not integrated into the genome.

As used herein, the term "co-transformed" refers to a process by which two or more recombinant DNA constructs or vectors are introduced into the same cell. "Co-transformed" may also refer to a cell into which two or more recombinant DNA constructs
25 or vectors have been introduced.

As used herein, the term "sequence preferential binding" refers to the binding of a molecule to DNA in a manner that indicates a preference for binding to a certain DNA sequence relative to others.

As used herein, the term "sequence specific binding" refers to the binding of a
30 molecule to DNA in a manner that indicates a strong binding preference for a particular DNA sequence.

As used herein, the term "sequence-dependent binding" refers to the binding of molecules to DNA in a manner that is dependent upon the target nucleotide sequence. Such binding may be "sequence-preferential" or "sequence-specific".

As used herein, the term "inhibit binding" relative to the effect of a given concentration of a particular compound on the binding of a transcriptional regulatory protein to its DNA response element refers to a decrease in the amount of binding of the transcriptional regulatory protein to its DNA response element relative to the amount of binding in the absence of the same concentration of the particular compound, and includes both a decrease in binding as well as a complete inhibition of binding.

As used herein, the terms "compound", "molecule", "ligand" and "inducer" are used interchangeably and refer to molecules or ligands characterized by sequence-preferential or sequence-specific binding to DNA at a sequence which is adjacent (*i.e.*, flanking), overlapping, or the same as, the DNA binding site for a transcriptional regulatory protein.

As used herein, the terms "modulate" and "modify" are used interchangeably and refer to a change in biological activity. Modulation may relate to an increase or a decrease in biological activity, binding characteristics, or any other biological, functional, or immunological property of the molecule.

As used herein, the term "regulate gene expression" relative to a promoter of the invention means the promoter has the ability to increase or decrease the expression of, and may be used to modulate the level of expression of a gene operably linked thereto.

As used herein, the terms "native", "natural" and "wild-type" relative to a particular nucleic acid sequence, trait or phenotype refers to the form in which that nucleic acid sequence, trait or phenotype is found in nature.

As used herein, the term "exposure of said cell" relative to a cellular factor or compound which may interact with a cell that comprises a regulatory promoter sequence of the invention refers to both external and internal exposure. In the case of exposure to a cellular factor, the factor may be native (endogenous) or exogenously provided.

II. Regulated Gene Expression using Promoters of the Invention

The promoter elements of the present invention find utility in the regulated expression of genes, both native and heterologous.

In order to accomplish such regulated gene expression the regulatory components of a promoter of interest must be identified and characterized.

This is accomplished by the combination of identifying and characterizing the sequence of promoter components involved in the control of gene transcription and correlating such structural (sequence) components with a functional analysis of gene expression using the promoter.

In general, to determine if a particular DNA sequence is involved in the regulation of gene expression, a putative regulatory sequence is selected and operably linked to a reporter sequence in a heterologous nucleic acid construct which is then introduced into a cell, then the reporter activity is determined. For example, the expression of luciferase, a
5 gene originally isolated from the firefly that emits a photon in the presence of the substrate luciferin and ATP is easily monitored using a luminometer.

In one application of such regulated gene expression, compound binding sequences, located in the vicinity of the DNA response element for a transcriptional regulatory protein are incorporated into promoter constructs and used to regulate
10 expression of a gene under the control of a given promoter. The compound binding sequences may be native or introduced.

In another exemplary embodiment, the binding of a compound in the vicinity of (i.e., directly, adjacent to, or overlapping) the DNA response element for a transcriptional regulatory protein provides a means to modulate transcription of a native gene operably
15 linked to the DNA response element.

The identification and characterization of the regulatory regions of a promoter and using that information to design constructs which have one or more compound binding sequences in the vicinity of the DNA response element for a given transcriptional regulatory protein provides a means to regulate expression of native genes *in vivo* in a
20 cell. In such cases, providing the compound to a cell and the binding of the compound to a compound binding sequence within the regulatory region of a promoter results in regulated expression of a native gene under the control of that promoter.

In another exemplary embodiment, the binding of a compound in the vicinity of (i.e., directly to, adjacent, or overlapping) the DNA response element for a transcriptional regulatory protein provides a means to modulate transcription of a transgene operably
25 linked thereto. Any DNA binding compound that modulates the binding of a transcriptional regulatory protein to its DNA response element can be utilized to modulate expression of a transgene under the control of a promoter based on the present invention. The presence of a native or introduced compound-binding sequence in the vicinity of the DNA
30 response element for a transcriptional regulatory protein permits a wide selection of compounds effective to regulate the expression of genes operably linked to a promoter wherein the promoter includes the DNA response element.

It will be understood that a promoter of the invention may include a minimal promoter element and an introduced DNA response element, or the promoter itself may
35 contain a DNA response element. In general, the DNA response element or regulatory

promoter sequence refers to the sequence to which transcriptional regulatory proteins bind and may or may not be considered part of the promoter.

5 In some cases, the nucleic acid sequence in the vicinity of the DNA response element will include a sequence that is the preferred or specific binding site for a DNA binding compound.

In other cases, the promoter sequence in the vicinity of the DNA response element will be modified to include one or more preferred binding sequences for a DNA-binding compound resulting in a regulatable promoter construct.

10 For example, the promoter may include one or more compound binding sequences in the vicinity of the DNA response element, as exemplified by an 8 to 20 or more bp "AT-rich" sequence which is a preferred binding preferred binding sequence for the netropsin dimer, "21x".

15 A transcriptional regulatory protein/DNA response element/compound binding sequence combination together with a compound which preferentially or specifically binds to that compound binding sequence may be useful for regulated expression of a transgene under the control of any of the promoters described herein. However, in some cases, the transcriptional regulatory protein/DNA response element/compound binding sequence combination and the compound which preferentially or specifically binds to that compound binding sequence is specific to a given promoter.

20 Compounds for use in regulating expression of a transgene under the control of a particular promoter are generally pre-selected based on the ability to regulate the expression of a transgene under the control of a given promoter.

25 Exemplary pre-screening assays include, but are not limited to, DNA binding assays; protein displacement assays; DNA footprinting, etc. As set forth herein, such assays may be carried out using various techniques known in the art.

30 In one embodiment, compounds for use in regulating gene expression are pre-selected for DNA-binding and transcriptional regulatory protein displacement. Exemplary pre-screening assays include various forms of the Merlin™ assay, e.g., co-owned U.S. Pat. Nos. 5,306,619, 5,693,463, 5,716,780, 5,726,014, 5,744,131, 5,738,990, 5,578,444, 5,869,241, expressly incorporated reference herein.

In another embodiment, compounds are pre-selected in a nucleic acid ligand interaction assay, such as that described in PCT Publication No. WO 00/15848, or another nucleic acid binding assay known to those of skill in the art.

III. Promoter Isolation and Characterization

The promoters described herein were isolated and characterized employing methods generally known in the art, including, but not limited to, walking upstream from the coding sequence of a known gene to identify regulatory sequences, analysis and
5 characterization of previously identified promoter sequences by linker scanner mutation and site directed mutagenesis.

In some cases, promoter sequences are obtained by walking upstream in a PCR-accessible genomic library (e.g., using GenomeWalker, Clontech) using primers designed based on a known coding or other sequence. Sequential upstream walks are used to
10 generate longer DNA sequences, extended at the 5' end in order to identify regulatory sequences. The sequence obtained from a first walk is used to design primers for a second upstream walk, etc.

In other cases, the full sequence of a particular promoter for which the regulatory sequences are described herein, was known in the art. However, in such cases the
15 characterization of the promoter was not known prior to the present invention. In other words, the present invention represents identification and characterization of sequences critical to promoter activity.

In some cases, a series of promoters were constructed by introducing mutations in one or more regions of the promoter sequence followed by evaluation of the activity profile
20 of the mutated promoters.

IV. Promoter Activity Screening

Exemplary assays for evaluation of promoter activity include, but are not limited to, DNA binding assays useful for detection of the binding of a transcriptional regulatory
25 protein to the DNA response element of a promoter; protein displacement assays, such as gel mobility shift assays, competitive binding assays and DNA footprinting, etc. Such assays may be carried out using various techniques known in the art.

Gel mobility shift assays may be used to determine the effect of a compound on the binding of a transcriptional regulatory protein to the DNA response element within a
30 given promoter, based on the change in size (and corresponding mobility on a gel) of the DNA/protein complex relative to the DNA alone.

DNA footprinting may be used to characterize the DNA response element of a given promoter for a transcriptional regulatory protein based on the stability of a promoter/transcriptional regulatory protein complex to nuclease degradation. The main application
35 of this approach has been for DNA footprinting (a method used to identify the DNA

sequence to which particular transcriptional regulatory proteins bind). Various techniques for DNA footprinting are known in the art.

Competitive hybridization-stabilization binding assay (HSA)

5 The binding preference of compounds to critical sequences in the promoters of the invention has been examined using a competitive hybridization-stabilization binding assay (HSA). In the HSA, a nucleotide sequence of interest is represented in an oligonucleotide duplex, and the duplex is tested for its ability to compete with an indicator oligonucleotide duplex which is known to bind the test molecule with a certain degree of affinity. The indicators may be rich in AT bases and labeled with either a fluorescent probe or a
10 quencher moiety on each of the two strands. The binding of the compound to the indicator stabilizes the duplex formation allowing the fluorescence to be quenched. If the compound prefers the test sequence (competitor) more than the indicator, it is less available to stabilize the indicator duplex and thus quenching is reduced. Therefore, a higher fluorescence signal implies a higher degree of binding preference to the test
15 sequence relative to the indicator.

In one example involving the cyclin D1 promoter, the hybridization stabilization assay employs a 12bp DNA duplex as an indicator for binding, wherein one strand of the duplex (CTTTATTATTTT) is 5' labeled with fluorescein, and the complementary strand is
20 5' labeled with a dabsyl quenching molecule (AAAATAATAAG-3'). When the two strands are mixed together with a DNA-binding molecule, which can stabilize the duplex form, the signal from the fluorescein is quenched by the dabsyl on the complementary strand. Various cold competitor duplexes can then be added to see whether they provide preferred binding sites for the DNA-binding compound. If the competitor DNA, binds the DNA-binding molecule, the DNA-binding molecule is titrated away from the indicator
25 duplex resulting in destabilization of the indicator duplex and as the strands separate, quenching is diminished and fluorescence increases.

Promoter-walk analysis Typically, a full promoter sequence is presented in blocks of 15 nucleotides as the competitor in a HSA. To cover the entire promoter, stretches of 15-mers are blocked in an overlapping manner so that neighboring blocks differ by two
30 nucleotides. An increase in fluorescence in the HSA implies a preference in binding.

RNase protection The effect of a modified DNA sequence on RNA transcription may be measured directly using an assay that includes either RNase protection or Northern analysis to monitor mRNA levels. RNase protection is a method of quantitating RNA based on its ability to form a nuclease resistant hybrid with a labeled probe. With
35 more RNA, more probe can be protected. If only part of the probe hybridizes to the RNA

of interest (*i.e.*, the probe has 5' or 3' regions that are not homologous to the RNA of interest), then only part of the probe is protected. The protected probe and the intact probe will migrate at different rates when subjected to gel electrophoresis. Protection of a fragment of a unique and predictable size indicates specificity. The probe can be either
5 an RNA or a DNA probe.

Linker Scanning Mutagenesis is a procedure in which short sequences of a DNA (*i.e.* sequences 5' to a known promoter) are substituted with DNA containing one or more restrictions sites, usually using a PCR based mutagenesis approach.

Reporter Constructs

10 A reporter construct is generally used in a cell-based *in vitro* assay to confirm promoter activity and the regulated expression of a transgene by a promoter.

In one embodiment, the luciferase reporter gene is used to evaluate regulatable gene expression *in vitro* in cell culture. However, any reporter gene known to those of skill in the art may also be used. It is preferable that expression of the selected reporter
15 gene be readily detected and quantitated in order to quickly evaluate numerous modified regulatory sequences. Such reporter constructs provide a means to evaluate the ability to regulate gene expression by a given promoter, *e.g.*, by targeting with a DNA-binding compound. Once the ability of a given promoter to regulate gene expression has been demonstrated in a cell-based assay using a reporter construct, the genetic construct may
20 be readily modified to include a transgene of interest, such as a therapeutic gene, recombinant protein-encoding gene or drug resistance gene, in place of the reporter gene. Such modifications may be made using techniques routinely employed by those of skill in the art.

25 V. Cyclin D1 Promoter

Cyclin D1 (CCND1) is a regulatory protein overexpressed in many carcinomas. Cyclin D1 acts by binding to and regulating the cyclin dependent kinases CDK4 and CDK6. CCND1 gene expression is low in quiescent cells (in G₀) but is induced as cells respond to growth factors and enter the cell cycle leading to an increase in active cyclin
30 D1-CDK4/CDK6 complexes.

Rapid cell cycling irrespective of appropriate growth signals and failure to respond to growth inhibition signals such as contact inhibition are characteristics of cancer cells. Inappropriate expression of cyclin D1 during chromosomal inversion, translocation or amplification has been characterized in a variety of tumor cells (Hall *et al.*, 1996; Sherr,
35 1996). Cyclin D1 gene overexpression is also seen in many tumors without gross

chromosomal rearrangements or amplification of the cyclin D1 gene. In fact, overexpression of cyclin D1 is seen in 50% of primary breast carcinomas, in 30% of adenocarcinomas of the colon cells (Hall *et al.*, 1996), in familial adenomatous polyposis (Zhang *et al.*, 1997) as well as in many cases of pancreatic cancer (Gansauge *et al.*, 5 1997).

In addition, transgenic mice that overexpress the cyclin D1 gene in mammary epithelium show mammary hyperplasia and develop mammary adenocarcinomas (Wang *et al.*, 1994). Overexpression of cyclin D1 in cultured cells results in early phosphorylation of pRB retinoblastoma protein (Sherr, 1993), shortening of the G1 phase and makes 10 these cells growth factor independent (Jiang *et al.*, 1993; Quelle *et al.*, 1993; Resnitzky *et al.*, 1994). When injected into nude mice these cells produce tumors (Jiang *et al.*, 1993).

The link between inappropriate expression of cyclin D1 and tumorigenesis indicates that cyclin D1 is a good target for therapeutic intervention. Cyclin D1 antisense molecules have been shown to reduce the neoplastic phenotype of human esophageal, 15 colon and pancreatic cancer cells overexpressing cyclin D1 in culture as well as the ability of these cells to produce tumors in mice (Zhou *et al.*, 1995; Arber *et al.*, 1997; Kommann *et al.*, 1998). In these studies antisense technology was used to specifically inhibit cyclin D1 mRNAs.

Accordingly, regulated expression of cyclin D1 finds utility in cancer and other 20 therapies. The present invention is based on CCND1 promoter analysis and identification of DNA response elements within the cyclin D1 promoter that are involved in regulation of gene expression, when under the control of the cyclin D1 promoter.

The human CCND1 gene has been previously cloned and sequenced (Motokura *et al.*, 1991; Withers *et al.*, 1991; Xiong *et al.*, 1991). An upstream promoter sequence of 25 the CCND1 gene has also been cloned and sequenced (Herber *et al.*, 1994a, 1994b; Philipp *et al.*, 1994). The CCND1 promoter sequence may be found in GenBank at Locus HUMPRDA1A (Motokura *et al.*, 1993).

Potential Sp1, E2F, CRE, Oct1, Myc/Max, AP-1, Egr, NFκB, STAT5, Ets, PRAD and TCF/LEF sites have been previously identified in the cyclin D1 promoter (Motokura *et al.*, 1993; Herber *et al.*, 1994; Philipp *et al.*, 1994; Hinz *et al.*, 1999; Matsumura *et al.*, 30 1999; Shtutman *et al.*, 1999; and Tetsu *et al.*, 1999). Several of these sites have been demonstrated to play a role in cyclin D1 regulation in various cell lines (Philipp *et al.*, 1994; Albanese *et al.*, 1995; Watanabe *et al.*, 1996; Yan, *et al.*, 1997; Watanabe *et al.*, 1998; Beier *et al.*, 1999; Hinz *et al.*, 1999; Matsumura *et al.*, 1999; Shtutman *et al.*, 1999; 35 and Tetsu *et al.*, 1999).

The CRE region of the CCND1 promoter (nucleotides -52 to -45) has previously been identified as important for cyclin D1 expression in various cell types (Beier *et al.*, 1999; Tetsu *et al.*, 1999; Phillip *et al.*, 1994; Lee *et al.*, 1999). In particular, the CRE promoter element has been demonstrated to be required for basal expression of the cyclin D1 gene in MCF7 cells.

Although the prior art includes some analysis of the cyclin D1 promoter, the prior art does not indicate appropriate targets for regulated gene expression using the cyclin D1 promoter. One aspect of the present invention is directed to modulating cyclin D1 expression in cancer cells that overexpress the gene, based on particular sequences identified as targets for regulation.

Analysis of transcription factor binding sites in the cyclin D1 promoter was carried out to identify portions of the cyclin D1 promoter that can be used to regulate the expression of a gene operably linked to the cyclin D1 promoter. An extensive promoter analysis was performed in a variety of different cancer cell lines that overexpress cyclin D1 and important transcription factor binding sites were identified, as detailed in Example 1.

A 1900-bp fragment of the human cyclin D1 promoter was PCR amplified from genomic DNA and subcloned into the vector pGL3-basic (Promega) to form a reporter construct. A series of modified promoters were made and promoter activities compared to that of the full-length (-1745) cyclin D1 promoter (Fig. 4) following transfection into asynchronous MCF7 human breast carcinoma cells, which overexpress cyclin D1, in order to identify important regulatory regions of the promoter. Some constructs were further evaluated in another cyclin D1 overexpressing breast carcinoma cell line (ZR75); in a breast cell line (HMEC) that expresses cyclin D1 normally; in a cyclin D1 overexpressing colon cancer cell line (HCT116); and an overexpressing pancreatic cancer cell line (PANC-1).

The various modified promoter constructs include 5' deletions, site-directed mutagenesis of the AP1, CRE, E2F, SP1 and Oct1 sites, and mutants prepared using linker-scanning mutagenesis of the proximal promoter generated using the QuickChange mutagenesis system.

The results provided herein indicate that the regulatory sequences presented as SEQ ID NO: 5, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:9 find utility in regulating the expression of autologous or heterologous genes operably linked to a cyclin D1 promoter comprising one or more of the regulatory sequences.

VI. CD40 ligand (CD40L)

CD40 ligand or CD40L (also referred to as gp39, CD154, TRAP or T-BAM) plays a critical role in T cell dependent humoral immune responses. CD40L interacts with CD40, which is expressed on the surface of antigen presenting cells (APCs; Ochs *et al.*, 1994; Foy *et al.*, 1996; Grewal *et al.*, 1996). Antigen presenting cells process antigens and present them on their surface in combination with major histocompatibility complex (MHC) molecules. This provides one signal necessary for T cell activation and recognition of an antigen-MHC complex by the T cell receptor which triggers the transient expression of the membrane bound cytokine CD40L on activated CD4+ helper T cells. Interaction between CD40 and CD40L is necessary for B cell activation and isotype switching. The binding of CD40L to CD40 induces the expression of the costimulatory molecules B7.1 (CD80) and B7.2 (CD86) on APCs which in turn, bind to CD28 on T cells providing the second costimulatory signal necessary for T cell activation. Engagement of the T cell receptor by antigen-MHC in the absence of the second signal produces T cell anergy. A human genetic defect in the CD40L gene causes the X-linked immunodeficiency disorder called hyper-IgM syndrome (Allen *et al.*, 1993; Aruffo *et al.*, 1993; DiSanto *et al.*, 1993; Korthauer *et al.*, 1993). Affected individuals either fail to express CD40L or express CD40L incapable of binding to CD40 resulting in significantly reduced T cell-dependent humoral immune responses and an absence of isotype class switching.

Targeting the CD40L promoter therefore has implications to a number of autoimmune disorders, including but not limited to, multiple sclerosis (MS), systemic lupus erythematosus (SLE), graft-vs-host disease (GVHD) and rheumatoid arthritis. (See, e.g., Buhlmann *et al.*, 1996; Biancone *et al.*, 1999). In addition, there is evidence that inhibiting CD40L expression can contribute to long term transplantation tolerance (Larsen *et al.*, 1996; Kirk *et al.*, 1997; Hancock *et al.*, 1998; Niimi *et al.*, 1998). Further, targeting CD40L with specific monoclonal antibodies has been shown to increase the effectiveness of adenovirus vector based gene therapy (Yang *et al.*, 1996; Kay *et al.*, 1997).

The human CD40L gene has been cloned (Graf *et al.*, 1992; Hollenbaugh *et al.*, 1992; Spriggs *et al.*, 1992; Gauchat *et al.*, 1993; Shimadzu *et al.*, 1995). The CD40L promoter sequence contains several potential transcription factor binding sites: AP-1 (1570 to 1577; 1867 to 1938), GMCSF (1040 to 1145; 1343 to 1350; 1689 to 1696; 1840 to 1862), α IRE (1291 to 1295; 1359 to 1366; 1397 to 1404; 1589 to 1593; 1701 to 1705; and 1803 to 1807), TCF1 (1603 to 1606; 1731 to 1736), GATA-1 (1643 to 1647),

CRE 2(1209 to 1216), γ INF2 (1188 to 1195), NF-IL6 (815 to 819) and NF κ B (737-743) as identified by sequence analysis (GenBank Accession No. D31793).

In order to characterize the CD40L promoter, the full-length human CD40L promoter from -1860 to +49 (SEQ ID NO:1) was PCR amplified and cloned into the firefly luciferase reporter plasmid pGL3-basic, as detailed in Example 2. A series of 5' CD40L promoter deletions and specific mutations were prepared, PCR amplified and cloned into the firefly luciferase reporter plasmid pGL3-basic, the authenticity of all clones verified by DNA sequencing and promoter activity of the 5' deletion constructs compared to that of the full-length (-1860) CD40L promoter following transfection into normal expanded T cells and activation with PMA and ionomycin (Example 2).

The results indicate that at least four regions of the CD40L promoter are critical to expression in activated T cells, including a site near nucleotide position -306, the specific mutation of which resulted in a 4-fold down regulation of CD40L promoter activity factor binding at the site. (See Example 2)

A second promoter region that plays a role in controlling CD40L expression is the sequence between nucleotides -230 and -211 (SEQ ID NO:13), based on deletion of the region which resulted in a 6.7-fold reduction in promoter activity.

A third region important to CD40L promoter expression in activated normal human T cells is found between -230 and -196 (SEQ ID NO:14), based on deletion of the -230 to -211 region, which resulted in an 6.7-fold downregulation of CD40L promoter activity, and site specific mutations of -220 to -215, -214 to -209, -208 to -203 or -202 to -197, which resulted in a 2.5 to 4-fold down regulation of promoter activity. A T cell-specific, sequence-specific factor was demonstrated to bind in the -206 to -201 region based on the results of *in vivo* footprinting analysis.

A fourth region identified in the CD40L promoter as important for expression in activated normal human T cells is found between -77 and -40 (SEQ ID NO:15) based on the expression level of deletion mutants, wherein an internal deletion of -72 to -49 or -61 to -40 resulted in a 25-fold or 40-fold downregulation respectively. In addition, specific mutations in the composite AP-1/-66 NF-AT site together with a previously unidentified site located between -48 and -54 indicates a contribution to transcriptional activation through the -48 to -54 site.

It will be appreciated that some CD40L promoter regions may bind more than one transcription factor, as further discussed in Example 2. It will further be appreciated that targeting a DNA-binding compound to a regulatory region of the CD40L promoter

described herein, provides a means to inhibit CD40L promoter-mediated transcription through modulation of transcription factor-DNA interactions.

The results provided herein indicate that the regulatory sequences presented as SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15 find utility in regulating the expression of autologous or heterologous genes operably linked to a CD40L promoter comprising one or more of the regulatory sequences.

VII. Hepatitis B (HBV)

Viral induced Hepatitis B in humans is caused by infection with HBV, which is estimated to have infected 300 million people worldwide. A small but significant portion of the infected individuals develop severe pathologic consequences, including chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma, with one million deaths per year caused by HBV infection worldwide.

Vaccination is an effective preventive measure, however, there is no cure for the disease, and at present there is no effective treatment specific to acute hepatitis B. Currently, chronic hepatitis B is treated with interferons (*i.e.*, interferon-alpha) and nucleoside analogs (*i.e.*, lamivudine "3TC").

HBV was initially cloned in the 1970s (Robinson *et al.*, 1974; Sattler *et al.*, 1979; Summers *et al.*, 1975). Human hepatoma cell lines (HepG2 and HuH6) have HBV stably integrated within the cellular genome. These cells can support HBV replication and release virus-like particles into the tissue culture media. See, *e.g.*, See MA *et al.*, 1987; Lander *et al.*, 1997; Sudo *et al.*, 1996.

HBV is a DNA virus which has a genome consisting of a relaxed, circular, partially duplex DNA species of 3.2 kb. Every nucleotide in the genome is within a coding region, and over half of the sequence is translated in more than one opening reading frame. Several promoters have been identified, driving expression of (a) pre-core proteins, core proteins and polymerase (core promoter); (b) large S surface protein (pre-S1 promoter); (c) medium and small S surface proteins (S promoter); and (d) X protein (X promoter). The core protein encapsulates the viral genome and polymerase, the various S surface proteins make up the protein coat, and the function of the X protein has not been determined.

Characterization of the core promoter, which directs the transcription of two greater than genome size messenger transcripts, has been described (for reviews, see Ganem D., in *FIELD VIROLOGY* 3rd Ed. 1996 and Kann M. and Gerlich W., in *Viral Hepatitis*, 2nd Ed). One of these mRNAs, the pregenomic transcript encodes both the core structural

protein and the viral polymerase as well as template for replication of the negative strand viral DNA. The other 3.5 kb mRNA, the pre-core message, is translated and modified into the soluble viral e antigen. Binding sites for hepatocyte nuclear factors, C/EBP, and Sp1 have previously been described in the core promoter region (as reviewed in Ganem D., in
5 FIELD VIROLOGY, 3rd Ed. 1996 and Kann M. and Gerlich W., in VIRAL HEPATITIS, 2nd Ed). The hepatocyte nuclear factors, HNF3 and HNF4, are believed to be important for the liver tropism of HBV. Additional transcription factor binding sites such as C/EBP and Sp1 have been described.

A characterization of three HBV promoters is provided herein; a core promoter
10 (SEQ ID NO:16, Fig. 1A), a pre-S1 promoter (SEQ ID NO:22, Fig. 1B), and the HBV-X promoter (SEQ ID NO:25, Fig 3).

The HBV promoters described herein find utility in regulated gene expression which is specific to liver cells:

Analysis of the effect of modification of various sequence components of the HBV
15 core, preS1 and X promoters was carried out to identify portions of the promoters that can be used to regulate the expression of a gene operably linked to the HBV core, preS1 or X promoter, respectively, as detailed in Example 3.

Luciferase reporter activities of wild type core, X, and preS1 promoter constructs and various modifications thereof were evaluated by transient transfection experiments in
20 cell lines of hepatic origin such as HepG2, Huh7, 22.1.5, and HepAD38.

HBV Core Promoter

Three regions of interest were identified in the linker scanning analysis of the HBV core promoter. The TATA box, HNF4 (SEQ ID NO:18) and proximal HNF3 (SEQ ID
25 NO:17) sites were identified as the control elements most critical to core promoter activity. As further described in Example 3, three regions of the HBV core promoter, domain 5; domain 8/9 and domain 13 appear to be in the vicinity of cis-elements (HNF-4/HNF-3, HNF-3/Sp1, and the TATA box, respectively) reported in the literature. The results of expression studies presented in Table 9 suggest that domain 8 (SEQ ID NO: 19); domain
30 8/9-1 (SEQ ID NO:20); and domain 13 (SEQ ID NO: 21) are involved in transcriptional activation and that those sequences find utility in regulating the expression of autologous or heterologous genes operably linked to an HBV core promoter comprising SEQ ID NO:20 and/or SEQ ID NO:21.

preS1 Promoter

A luciferase reporter construct was generated using a full-length copy of the HBV genome with the preS1 promoter positioned immediately upstream of the luciferase reporter gene and site-directed mutagenesis was performed to generate four mutants in known transcription factor binding sites and linker scanner mutants. The mutagenized constructs were transiently transfected into Hep3AD38 and tested for promoter activity, as described above. As detailed in Example 3, a known transcription factor binding site, designated HNF1 was found to be critical to preS1 promoter activity.

The results provided herein indicate that the regulatory sequences presented as SEQ ID NO: 23 and SEQ ID NO:24 find utility in regulating the expression of autologous or heterologous genes operably linked to an HBV preS1 promoter comprising one or both of the regulatory sequences.

HBV X Promoter

The HBV X promoter was analyzed by deletion and linker scanning experiments similar to those described for the core promoter.

A luciferase reporter construct was constructed with a full-length copy of the HBV genome and the HBV X promoter positioned immediately upstream of a reporter coding sequence. Promoter constructs were prepared with successive blocks of 21 base pair mutations in the HBV X promoter or known transcription factor binding sites. Mutant constructs were transfected into the hepatoma-derived HepG2 and HepG2 cell lines stably transfected with HBV: 22.1.5 and HepAD38, and the expression of the luciferase reporter gene analyzed to determine HBV promoter activity, as detailed in Example 3. Mutations in domains 3, 4 and 6 as well as double mutants (domains 3 + 6 and domains 4 + 6), yielded the greatest reduction in activity. Additional HBV-X promoter reporter constructs were made with mutations in various known transcription factor binding sites and evaluated for luciferase reporter activity suggesting that domains 18 and 19 are also important for activity of the HBV X promoter.

The results provided herein indicate that the regulatory sequences presented as SEQ ID NO: 26, SEQ ID NO:27 and SEQ ID NO:28 find utility in regulating the expression of autologous or heterologous genes operably linked to an HBV X promoter comprising one or more of the regulatory sequences.

VIII. Vancomycin-Resistant Enterococci (VRE)

Recently, a rapid increase in the incidence of infection and colonization with vancomycin-resistant enterococci (VRE) has been reported. The observed resistance is of concern due to (1) the lack of effective antimicrobial therapy for VRE infections because most VRE are also resistant to drugs previously used to treat such infections, *i.e.*, penicillin and aminoglycosides (CDC, 1993; Handwerger *et al.*, 1993); and (2) the possibility that the vancomycin-resistant genes present in VRE can be transferred to other gram-positive microorganisms.

Although enterococci can be part of the normal flora of the gastrointestinal and female urogenital tracts, recent studies indicate that enterococci can be transmitted directly in the hospital setting. (See, *e.g.*, Boyce, *et al.*, 1994.) *Enterococci* have been recognized as a cause of nosocomial infection and some strains are resistant to multiple antimicrobial drugs. The most common enterococci-associated nosocomial infections are urinary tract infections, post-surgical infections and bacteremia (Murray, 1990; Moellering RC Jr., 1992; Schaberg *et al.*, 1991).

Vancomycin has been used extensively to treat *Enterococcus* infection since the late 1970s. Recently, a rapid increase in the incidence of infection and colonization with vancomycin-resistant enterococci (VRE) has been reported.

Resistance to vancomycin and other glycopeptide antibiotics has been associated with the synthesis of a modified cell-wall precursor, terminating in D-lactate which has a lower affinity for antibiotics such as vancomycin.

Typically gram positive bacterial cell wall synthesis involves assembly, membrane transport, incorporation into the cell wall and cross linking of a pentapeptide precursor molecule as part of the process of peptidoglycan formation. Vancomycin functions by forming a complex with the peptidyl-D-ala-D-ala precursor, thereby inhibiting precursor transport by transglycosylases and incorporation into the peptidoglycan, and weakening the bacterial cell wall. Type A high-level vancomycin resistance is achieved via an operon that replaces the C-terminal D-ala with D-lac, such that vancomycin binding is inhibited (Walsh C, 1999).

The operon is controlled by a two component regulatory system that consists of a sensor protein, VanS and a cytoplasmic response regulator, VanR.

VanS is a two domain transmembrane signaling kinase which undergoes an autophosphorylation at histidine residue (H164). Phospho-VanS in the presence of ATP can undergo phosphotransfer to an aspartate residue on VanR (2). Studies have shown that phospho-VanR binds with high efficiency to P_{vanH} and enhances transcription of the

genes necessary for vancomycin resistance (Haldimann *et al.*, 1997; Holman *et al.*, 1994).

The polysystronic message that gives rise to the genes necessary for high level inducible vancomycin and teichoplanin resistance consists of vanH, A, X, Y and Z. The
5 vancomycin resistance enzyme VanH is an alpha-ketoacid dehydrogenase that stereospecifically reduces pyruvate to D-lactate, which forms an integral part of the bacterial cell wall replacing the vancomycin target dipeptide D-alanine-D-alanine (Stoll *et al.*, 1998; Marshall *et al.*, 1999).

The present invention hypothesizes that a DNA binding molecule properly placed
10 within the vanH promoter will displace phospho-VanR and shut down transcription of inducible resistance genes, thus rendering the bacteria once again sensitive to vancomycin. Although the mechanism is not part of the invention, shutting down transcription of the resistance genes is preferable to shutting down the two component regulatory system due to potential cross talk between P_{vanH} and the response regulators
15 of other two component regulatory systems (Silva *et al.*, 1998).

It follows that regulating the expression of the vanH promoter has application to treatment of infectious disease.

DNA sequencing of the vanH promoter region from nine A type strains of VRE showed a high degree of sequence identity with the published A type regulatory region
20 (GenBank Accession N. M97297). A modified pAM401 plasmid (ATCC) was designed containing a VRE promoter sequence upstream of the luciferase gene in a background allowing for growth and maintenance in *E. coli* and *Enterococcus* species. (See Example 4.)

Site directed mutagenesis of the VRE promoter region was carried out by
25 systematically altering short 8 to 10 bp regions of the consensus promoter sequence, including the -35 consensus binding site within the phosphorylated VanR footprint (Arthur *et al.*, 1992). In addition, 20 linker scanning mutants designated M2-M21 were generated and nucleic acid constructs containing the VRE promoter sequence upstream of the luciferase gene were subcloned into the a pRLUC parent vector and transformed into *E.*
30 *coli*.

Each linker scanner promoter mutant was tested for activity, with both up- and down-regulation observed. The largest decrease was to 0% of wild type activity and the largest increase was 1737% of wild type activity. All mutants in the region reported to be
35 footprinted by phosphorylated VanR (M2-M8) showed decreased activity. Increased activity was observed in mutants spanning -30 to +20, suggesting the possibility of a

repressor binding site in this region. Mutants M8 and M9 were shown to consistently result in the highest luciferase activity of the mutants tested (Example 4).

5 The results provided herein indicate that the regulatory sequences presented as SEQ ID NO: 32, SEQ ID NO:33 and SEQ ID NO:34 find utility in regulating the expression of autologous or heterologous genes operably linked to a VRE promoter comprising one or more of the regulatory sequences.

IX. Androgen receptor

10 Prostate cancer is the most frequently diagnosed cancer in males in the United States. The cancer is treatable if diagnosed early, however, once the cancer metastasizes virtually all patients die within 12-18 months. Current treatments for metastatic prostate cancer involve targeting the androgen receptor (AR) using surgical or chemical means. The androgen receptor (AR) directly regulates gene expression when bound to androgens such as testosterone or dihydrotestosterone (DHT) and is required for
15 prostate maintenance. Once androgens are removed, genes regulated by the AR in the prostate are turned on or off resulting in programmed cell death or apoptosis.

The androgen receptor promoter from nucleotides -6000 to +1100 was cloned from genomic DNA by PCR using GenBank sequences for primer design. The amplified promoter sequence was subcloned into pGL3 basic (Promega) for subsequent transient
20 transfection and evaluation of luciferase expression. A large series of deletion constructs were made and tested following transient transfection, as detailed in Example 5.

The results of luciferase expression assays in the AR+ cell line, LNCaP, following transient transfection of various deletion constructs indicate the presence of a repressor binding site between nucleotides -2000 and -200 and multiple activator sites at
25 nucleotides -150 to -100 (homopurine stretch), -100 to -50 (SP1 site), and -50 to +1 (helix loop helix binding site).

More specifically, the results suggest that: (1) the 5' region of the homopurine region represents all of the activity from -150 to -100; (2) the region 3' of the downstream helix-loop-helix sequence contains another 2-fold of activity; and (3) the downstream
30 helix-turn-helix site contains 1.5-fold activity.

The present invention represents the identification of regulatory sequences of the AR promoter, examples of which are presented as SEQ ID NO: 64, SEQ ID NO:65 and SEQ ID NO:66, respectively. The regulatory sequences presented as SEQ ID NO: 64, SEQ ID NO:65 and SEQ ID NO:66 find utility in regulating the expression of autologous or

heterologous genes operably linked to an AR promoter comprising one or more of the regulatory sequences.

X. Her2

5 Her2 (human epidermal growth factor receptor2; c-erbB2, neu) is a tyrosine kinase growth factor receptor implicated in the metastatic growth of a subclass of breast cancers. Her2 overexpression occurs in up to 30% of patients with breast cancer and is associated with an especially aggressive form of the disease characterized by more rapid disease progression and shortened survival. Her2 can be overexpressed in breast
10 cancer cells, ovarian cancer cells as well as a variety of other cancer cells. Accordingly, regulated expression of Her-2 would be useful to modulating such overexpression.

A 2000-bp fragment of the human Her2 promoter was PCR amplified from genomic DNA using the following oligonucleotides. This purified fragment was subcloned into the vector pGL3-basic (Promega) at the NcoI and HindIII sites for use in
15 transient transfection luciferase expression assays in the breast carcinoma cell lines MCF-7 (low Her2 expression) and 2R75-1 (high Her2 expression).

The results of studies detailed in Example 6 indicate that the critical regulatory sites for the Her2 promoter lie between nucleotides -125 and -50. More specifically, sequences of interest for regulated expression of Her2 are a repressor sequence (SEQ
20 ID NO:70) downstream of the putative TATA box from -23 to -19; a complex regulatory region (SEQ ID NO:71) which includes both an activator and a repressor component; and a putative TATA box/ets site (SEQ ID NO:72).

The regulatory sequences presented herein as SEQ ID NO:70, SEQ ID NO:71 and SEQ ID NO:72 find utility in regulating the expression of autologous or heterologous
25 genes operably linked to a Her2 promoter comprising one or more of the regulatory sequences.

XI. Beta-lactamase (Bla) promoter

The extensive use of beta-lactam antibiotics has resulted in significant bacterial
30 resistance to such treatment. This resistance is generally mediated by lactamases in both gram-positive and gram-negative bacteria. More specifically, the beta-lactamase gene confers ampicillin resistance to a number of types of bacteria including *E. coli*. Recently, therapeutic approaches directed to overcoming such antibiotic resistance have been developed which include the delivery of a beta-lactam antibiotic in combination with a
35 beta-lactamase inhibitor.

Regulated expression of the beta lactamase gene provides another means to modify such antibiotic resistance. In order to determine which regions of the beta lactamase gene may be used to regulate beta lactamase expression, luciferase reporter constructs were prepared containing a beta-lactamase promoter sequence upstream of the luciferase gene.

Promoter mutants of the natural beta lactamase P3 bla promoter were generated by systematically altering the base pairs of the entire bla promoter sequence (from nucleotides -101 to +43).

Luciferase activities were measured in lysates prepared from *E. coli* XL1 Blue replicates. Mutants which exhibited significantly decreased luciferase activity included those with mutations in the -35 region (-41 to -30, M6); the -10 region (-17 to -6, M8); the start site (-5 to +7, M9); and +20 to +31 (M11). The luciferase activities of these constructs were reduced to 24%, 29%, 15% and 2% of wild type, respectively, as further described in Example 7.

Bla promoter linker scanner mutant constructs were generated by introducing 6 or 12 base pair mutations at different locations of entire bla promoter. Renilla luciferase reporter activities of the mutants measured and compared to the activity of the wild type pBla-Renilla luciferase construct.

The regulatory sequences presented herein as SEQ ID NO:77 and SEQ ID NO:78 find utility in regulating the expression of autologous or heterologous genes operably linked to a beta lactamase (bla) promoter comprising one or more of the regulatory sequences.

XII. Utility/Applications

The present invention is directed to isolation of various promoters, characterization of the promoters, and in particular characterization of regulatory elements of the promoters. The promoters described herein find utility in regulated gene expression and may function by interaction with natural cellular factors (e.g., transcriptional regulatory proteins) or by interaction with exogenously provided cellular factors or compounds.

The promoter may be a minimal or full length promoter. It will be understood that the promoter sequences described herein include minimal promoter elements alone or together with control sequences (also termed "transcriptional and translational regulatory sequences"), involved in expression of a given gene product. In general, transcriptional and translational regulatory sequences include, but are not limited to, the promoter sequence itself, the DNA response element for a transcriptional regulatory protein,

ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences.

The binding of a transcriptional regulatory protein to its corresponding DNA response element serves to regulate the expression of a gene under the control of a promoter operably linked to the promoter. Identification of sequences critical to such binding and regulation provides a framework for controlling the transcription and therefore the expression of a gene under the control of the promoter.

Accordingly, the promoter regulatory sequences described herein may be used to regulate the expression of genes operably linked to the relevant promoter. Such promoter regulatory sequences find utility in the design and construction of heterologous nucleic acid constructs and in the regulated expression of native genes.

The promoter regulatory sequences described herein may also be used in conjunction with a DNA binding compound to regulate the expression of a gene operably linked to the promoter.

In some cases, a given promoter may be regulated by a native factor, for example, the expression of a gene operably linked to a cell type-specific, developmentally regulated, or disease-specific promoter which promotes gene expression in certain tissues without affecting expression in other tissues may be regulated using the sequences described herein.

More specifically, the ability to regulate the expression of genes under the control of a cyclin D1 promoter has application to treatment of various cancers, including, but not limited to, breast cancers, colon cancers and pancreatic cancers.

Interaction between CD40 and CD40L is necessary for B cell activation and isotype switching. Therefore, regulation of the activity of the CD40L gene promoter finds utility in the treatment of various immunological disorders, such as autoimmune disease.

Regulated expression of genes under the control of the HBV-specific core, pre-S and X promoters find utility in the therapy of HBV disease and in the regulated expression of liver cell-specific genes.

Resistance to the antibiotic, vancomycin, which is used to treat *Enterococcus* infection has been associated with the vancomycin resistance enzyme VanH. Therefore, regulated expression of the vanH gene promoter has utility in treatment of *Enterococcus* infection.

Given that the androgen receptor (AR) is currently the target of numerous therapeutic strategies for treatment of prostate cancer, regulated expression of the androgen receptor gene promoter finds utility in the treatment of prostate cancer.

Her2 is a tyrosine kinase growth factor receptor implicated in the metastatic growth of a subclass of breast cancers as well as various other types of cancers. Therefore, regulated expression of the Her2 gene promoter has utility to treatment of cancer.

5 The β -lactamase gene confers ampicillin resistance to *E. coli*. Accordingly, regulated expression of the β -lactamase gene promoter is relevant to modulation of such antibiotic resistance.

10 The sequence information and functional characterization of the promoter regulatory sequences described herein can therefore be used to regulate the transcription of endogenous genes and transgenes (autologous and heterologous genes, respectively), in a variety of useful applications.

While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications and changes may be made without departing from the invention.

15

Material and Methods

Luciferase Assays Cells were washed once with PBS buffer, harvested in 1ml PBS, pelleted, and lysed with 100 μ l passive lysis buffer (Promega) at room temperature for 15-20 minutes. The cell lysates were centrifuged for 5 minutes, then 10 μ l of lysate is
20 added to 100 ml of luciferase assay reagent (Promega). Assays were carried out in a luminometer (EG&G Berthold). Luciferase activity is expressed as a rate of light units. Correction for transfection efficiency and variations in harvesting were done by cotransfecting an SV40 renilla-luciferase reporter gene (PRL-SV40) or a promoterless renilla-luciferase gene (PRL-Null) and determining the activity of the renilla luciferase
25 internal control in the same Dual Luciferase assay (Promega). After standardization with renilla luciferase activity, a relative luciferase activity was obtained, and the mean and standard deviation from triplicate wells were calculated. In general, transfections were repeated and reproduced in at least two independent experiments.

30

EXAMPLE 1

Cyclin D1 promoter analysis

The full-length human cyclin D1 promoter from -1745 to +155 (Fig. 4, SEQ ID NO:1) was PCR amplified and cloned into the firefly luciferase reporter plasmid pGL3
35 basic. A series of cyclin D1 5' promoter deletions were similarly constructed and cloned

into pGL3-basic. Mutant promoter constructs were assayed in MCF7 cells, a second cyclin D1 overexpressing breast carcinoma cell line, ZR75; a breast cell line that expresses cyclin D1 normally, HMEC; a cyclin D1 overexpressing colon cancer cell line, HCT116; and a cyclin D1 overexpressing pancreatic cancer cell line, PANC-1.

5

Construction of Plasmids

A 1900-bp fragment of the human cyclin D1 promoter was PCR amplified from genomic DNA using the following oligonucleotides: 5'-GCA CGC GTG CTA GCC AGC TGG GCC CTT GT-3' (SEQ ID NO:2) and 5'-ATC CAT GGA AGC TTT GGG GCT CTT CCT GGG CA-3' (SEQ ID NO:3). This purified fragment (SEQ ID NO:1), representing nucleotides -1745 to +155 relative to the transcription start site of the cyclin D1 promoter, was subcloned into the vector pGL3-basic (Promega) at the MluI and HindIII sites to form the reporter -1745D1/LUC. A series of 5' deletions were cloned using polymerase chain reaction of the native promoter plasmid as follows: a 5' deletion to -1590, a 5' deletion to -1440, a 5' deletion to -690, a 5' deletion to -545, a 5' deletion to -390, a 5' deletion to -245, and a 5' deletion to -90, using the PCR primer having the sequence presented as 5'-GCA CGC GTG CTA GCT GGA GCC TCC AGA GGG CTG T-3' (SEQ ID NO:4).

Promoter activities for the 5' deletion constructs were compared to that of the full-length (-1745) cyclin D1 promoter following transfection into asynchronous MCF7 human breast carcinoma cells, which overexpress cyclin D1. Deletion of cyclin D1 promoter regions between -1745 and -245, in the context of the full length promoter (-1745) had little effect on basal promoter activity in MCF7 cells.

Site-directed mutagenesis of the AP1, CRE, E2F, SP1 and Oct1 sites, and linker-scanning mutagenesis of the proximal promoter were generated using the QuickChange mutagenesis system and the parent -1745D1/LUC plasmid. Restriction enzyme analysis and DNA sequencing confirmed the integrity of these constructs.

Mutation of the E2F site {Motokura & Arnold, 1993} resulted in a construct which retained 63% of wild-type activity. Mutation of the CRE element resulted in a construct that retained 32% of wild-type activity, indicating that it is important to basal cyclin D1 expression in MCF7 cells.

-60 -37
AACAAACAGTAACGTCACACGGACT
TTGTTGTCATTGCAGTGTGCCTGA
CRE

In addition to MCF7 cells, mutant promoter constructs were assayed in another cyclin D1 overexpressing breast carcinoma cell line, ZR75; in a breast cell line that expresses cyclin D1 normally, HMEC; in a cyclin D1 overexpressing colon cancer cell line, HCT116; and an overexpressing pancreatic cancer cell line, PANC-1. The -1745 wild-type, the -10 deletion or various site-directed mutants of the cyclin D1 promoter were inserted into the promoter-less firefly luciferase plasmid (pGL3-basic) and co-transfected into various cells together with an SV40 promoter driven Renilla luciferase control plasmid. Firefly luciferase activity for each construct was normalized to Renilla luciferase activity and is shown relative to that of the full-length wild-type promoter (-1745).

Tissue culture

The human breast carcinoma cell lines MCF7 and ZR75 were maintained in DMEM/F12 medium with 10% fetal bovine serum, 10 µg/ml bovine insulin and antibiotics (penicillin/streptomycin). The human colon carcinoma cell line HCT116 was maintained in McCoy's medium with 10% fetal bovine serum and pen/strep. The human pancreatic cell line PANC-1 was maintained in DMEM/F12 with 10% fetal bovine serum and pen/strep. Human mammary epithelial cells (HMEC) were maintained in Epithelial Growth Media supplemented with bovine pituitary extract (50 µg/ml), hydrocortisone (500ng/ml), hEGF (10ng/ml), and insulin (5 µg/ml). All lines were maintained at 37°C, 5% CO₂. MCF7, ZR75, HCT116 and PANC-1 cells were purchased from the American Type Culture Collection. HMEC cells were purchased from Clonetics Corp.

Transient transfections

Cells were transiently transfected with LipofectAMINE (GIBCO Life Sciences) in triplicate in 6-well tissue culture plates (Corning, NY). Equal numbers of cells (3 x 10⁵/well) were seeded in each well 24 hours prior to transfection. Prior to transfection, cells were equilibrated in 800 µl fresh medium (OptiMEM with 5% FBS and pen/strep). Cells were transfected with 5 µg of reporter plasmid containing various different cyclin D1 promoter constructs in 200 µl transfection buffer. After 4 hours incubation with the transfection solution, cells were fed with 4 ml OptiMEM with 5% FBS and pen/strep. Cells were harvested 48 hours after transfection.

Analysis of Cyclin D1 promoter elements

Tables 1 and 2, below, show a summary of the results of deletion analysis studies of the cyclin D1 promoter in MCF7 cells. Various 5' deletions or site-directed mutations of

the cyclin D1 promoter were inserted into the promoterless firefly luciferase plasmid (pGL3-basic) and co-transfected into MCF7 cells human breast carcinoma cells, which overexpress cyclin D1 (Buckley, 1993), together with an SV40 promoter driven Renilla luciferase control plasmid. The length of each construct is indicated relative to the transcriptional start site (+1). Firefly luciferase activity for each construct was normalized to Renilla luciferase activity and is shown relative to that of the full-length wild-type promoter (-1745). The data are presented as the mean +/- SEM with a minimum of two independent transfections done in triplicate. Deletion of cyclin D1 promoter regions between -1745 and -245, in the context of the full length promoter (-1745), had little effect on basal promoter activity in MCF7 cells, even though several potential transcription factor binding sites have been previously identified in that region.

Cyclin D1 promoter constructs containing a mutation of the CRE in combination with a mutation of the -30 to -21 region resulting in severely compromised promoter activity in all of the cell lines tested. *In vivo* footprinting experiments carried out as described below demonstrate factor binding at both the CRE and the -30 sites in HCT116 cells.

In all cell lines tested, mutation of the CRE in constructs designated as CREbam and CRE4C5G reduced basal promoter activity considerably although the strongest effect was seen in MCF7 cells. The corresponding wild type sequence is presented as SEQ ID NO:7.

Mutation of the -30 to -21 site reduced basal cyclin D1 promoter activity in some cell lines, but not others. However, in all cell lines tested, mutation of the -30 to -21 site in combination with mutation of the CRE (construct CRE4C/-30-21) reduced basal promoter activity considerably and to a greater extent than did mutation of either site alone (Table 1). This suggests that both the CRE and the -30 to -21 sites are involved in transcriptional regulation of basal cyclin D1 promoter activity in all of the overexpressing cancer cell lines tested, as well as in HMEC cells which express normal levels of cyclin D1. The effects of mutations in various other regions of the cyclin D1 promoter are summarized in Table 2, below.

Table 1. Reporter Activity of Cyclin D1 Promoter Constructs

Promoter construct	MCF7 cells (% wild-type)	HCT116 cells (% wild-type)	ZR75 cells (% wild-type)	PANC-1 cells (% wild-type)	HMEC cells (% wild-type)
-1745,(wild-type)	100	100	100	100	100
-10	11 +/- 0.7	22 +/- 1.6	21 +/- 1.1	45 +/- 1.9	50 +/- 4.8
CREbam	32 +/- 1.7	46 +/- 3.3	64 +/- 6.8	52 +/- 7.5	50 +/- 2.1
3'CREm	102 +/- 7.1	86 +/- 8.8	92 +/- 6.4	89 +/- 4.3	74 +/- 2.1
5'CREm	160 +/- 3.6	120 +/- 15.6	N/D	99 +/- 6.3	N/D
CRE4C5G	33 +/- 5.0	69 +/- 5.1	54 +/- 8.3	52 +/- 4.9	N/D
-30- to 21	33 +/- 2.2	91 +/- 12.2	77 +/- 7.0	46 +/- 4.8	78 +/- 4.8
+1to +9	37 +/- 4.0	46 +/- 4.1	92 +/- 12.5	53 +/- 8.3	74 +/- 5.0
CRE4C/-30 to -2	11 +/- 1.3	30 +/- 4.5	38 +/- 11.4	40 +/- 6.9	26 +/- 1.7
CRE4C/+1 to +9	14 +/- 0.8	32 +/- 4.2	43 +/- 4.0	17 +/- 3.4	N/D

Table 2. Reporter Activity of Cyclin D1 Promoter Constructs

5

5' deletion	% WT activity
-1590	92 +/- 0
-1440	96 +/- 13.1
-690	79 +/- 3.5
-545	82 +/- 2.4
-390	81 +/- 7.1
-310	89 +/- 4.4
-245	78 +/- 7.6
-90	39 +/- 1.2
-10	11 +/- 0.7

Site-directed mutagenesis of the AP1, CRE, E2F, SP1 and Oct1 sites, and linker-scanning mutagenesis of the proximal promoter was carried out to determine the effect on promoter activity. The results indicate that mutation of the E2F site (Motokura *et al.*, 1993) resulted in a moderate decrease in activity, while mutation of the CRE element indicated that it is important to basal cyclin D1 expression in MCF7 cells.

A thorough analysis of the CCND1 promoter indicate that the CRE site at -52 is a critical site for cyclin D1 expression in HCT116 colon cancer cells, PANC-1 pancreatic cancer cells, MCF7 and ZR75 breast cancer cells and HMEC breast cells which express cyclin D1 normally. As shown in cyclin D1 Tables 3 and 4 below, mutation of bases -30 to -21 reduced basal promoter activity to 33% revealing another important and novel activator site for cyclin D1 expression in MCF7 cells. Mutation of bases +1 to +9 or +10 to +19 also reduced basal promoter activity, to 37% and 62%, respectively. A double mutant containing mutations in the CRE (SEQ ID NO:7) and the -30 to -21 site (SEQ ID NO:5) was constructed and transfected into MCF7 cells, resulted in only 11% of the activity of

the full-length wild-type promoter retained in all cell lines tested. A double mutant of the CRE in combination with the +1 to +9 site (SEQ ID NO:8) reduced activity to 14%.

Table 3. Reporter Activity of Cyclin D1 Promoter Constructs

5

Mutant Construct	Mutant Sequence	Wild Type Sequence	% Wild Type Activity
mAP1	AAAAAAAATACGCGTGAATGGA	AAAAAAAATGAGTCAGAATGGA	111 +/- 12.8
mAP1ds	TCACCAGTTCTTGGACTGT	TCAGAATGGAGATCACTGT	79 +/- 8.4
mE2F	GGAATTGGATCCCATTT	GGAATTTTCGGGCATTT	63 +/- 10.5
mOCT1	GGGGCGGGATCCTTCT	GGGGCGATTGCTTCT	92 +/- 7.7
mSP1	TGCGCTTTTAATTAAACCT	TGCGCCCGCCCCGCCCCCT	105 +/- 5.6
CREbam	CAGTGGATCCACACGG	CAGTAACGTCACACGG	32 +/- 1.7
CRE4C	CAGTAAGGTCACACGG	CAGTAACGTCACACGG	33 +/- 5.0
CRE4C5G	CAGTAAGCTCACACGG	CAGTAACGTCACACGG	33 +/- 5.0

Table 4 Reporter Activity of Cyclin D1 Promoter Constructs

Construct	Mutations in -30-21 region	% Wild Type Activity
WT/-1745	GAGTTTGT	100
-30 -21/-1745	TCTGGGATCC	33 +/- 2.2
-30 -26/-1745	TCTGGTGT	43 +/- 3.5
-25 -21/-1745	GAGTTGGCGG	34 +/- 4.7
-30 -28/-1745	TCCTTTGT	33 +/- 6.3
-28 -23/-1745	GATGGGATT	46 +/- 5.1
-23 -21/-1745	GAGTTTTC	138 +/- 16.4
10 bp 21x/-1745	GAGTTTTTTTAAG	87 +/- 11.4
8 bp 21x/-1745	GAGTTTAAAGAG	85 +/- 7.8

10

To examine the proximal promoter region in more detail, a series of site-specific mutations were made in 10 bp segments from -62 to +20, in the context of the full length promoter (-1745) in pGL3 basic. Luciferase activity was evaluated following transfection into MCF7 cells. The results as shown in Table 5 indicate that mutation of either the 10 bp immediately 5' of the CRE (construct 5'CREm), or of bases -20 to -11 increased promoter activity suggesting the presence of negative transcriptional regulatory sites in these regions. Site-directed mutagenesis of the -30 to -21 promoter region was carried out and constructs assayed in MCF7 cells. The assay results indicate that bases between -30 and -24 (GAGTTTT, nucleotides SEQ ID NO:6) are the most important for transcriptional activation from this site.

15

Table 5. Reporter Activity of Cyclin D1 Promoter Constructs

Proximal promoter mutant	% wild type activity
5'CREm	160 +/- 3.6
CRE4C	33 +/- 1.8
3'CREm	102 +/- 7.1
-40 -31	113 +/- 6.8
-30 -21	33 +/- 2.2
-20 -11	165 +/- 19.1
-10 -1	111 +/- 8.8
+1 +9	37 +/- 4.0
CRE4C/-30-21	11 +/- 1.3
CRE4C/+1+9	14 +/- 0.8

5 The identification of sequences important for transcriptional activation suggest that it is possible to specifically regulate endogenous cyclin D1 expression in tumor cells using a regulatory sequence of the promoter.

10 *In vivo* footprinting *In vivo* footprinting of the cyclin D1 promoter was carried out as described in Mueller PR and Wold B, Science, 246(4931):780-786, 1989. Transcription factor binding at the CRE and in the -30 to -21 region were evaluated by *in vivo* footprinting using dimethyl sulfate (DMS) or UV-light in HCT116 cells. The results of these studies indicate that the CRE is protected in both serum-starved and serum-stimulated cells, consistent with the mode of action for the CRE-binding protein CREB. (See, e.g., KWOK, 1994.) The results also indicate that a protein is binding to the -30 to
15 -21 region in HCT116 cells and that the site is protected in both serum-starved and serum-stimulated cells. The identity of the factor responsible for binding in the -30 to -21 region remains to be determined.

EXAMPLE 2

20 Identification Of CD40 Ligand (CD40L) Promoter Elements

The full-length human CD40L promoter from -1860 to +49 (SEQ ID NO: 9) was PCR amplified and cloned into the firefly luciferase reporter plasmid pGL3-basic. A 1920 bp element of the CD40L promoter region (from -1860 to +49, Figs. 5A-C) was PCR-amplified from genomic DNA (Clontech), using the following primers with 5' XhoI and 3'
25 HindIII sites to facilitate subcloning.

TTA TGA TAC CTC GAG GGG AGA GCA TTC AGG AAG ATG (SEQ ID NO:10); and
TGA ATC ACG AAG CTT TGG TAT CTT CTG GCA GAG AAG (SEQ ID NO:11).

30 All 5' deletions were generated in the same manner using primers containing a 3' HindIII and unique 5' XhoI sequence. Internal deletion and site directed mutants were

generated using Quick Change Mutagenesis (Stratagene) as per the manufacturer's recommendations. Mutant constructs were pre-screened by restriction digest of newly modified sites, and confirmed by sequencing. Mutant constructs were purified using a Qiagen endotoxin free isolation system.

5

Preparation of PBMC Peripheral blood mononuclear cells (PBMC) were purified from buffy coats by Ficoll-Hypaque centrifugation, washed 3 times in Dulbecco's phosphate buffered saline without calcium and magnesium, resuspended at 5×10^6 cells/ml in RPMI 1640 media (Gibco BRL), 15% FCS (Gibco BRL) and supplemented with 2mM L-glutamine (Gibco BRL), 1x Penicillin/Streptomycin (Gibco BRL) and 10% IL-2 (Hemagen Diagnostics), then plated in 12-well plates at 2mls/well. PBMC were then stimulated with TSST-1 (Toxin Technologies) at a final concentration of 50ng/ml. Cells were cultured at $3-3.8 \times 10^6$ cells/ml, cultured for one week, then subjected to Ficoll-Hypaque centrifugation, and plated in 12-well plates at 3mls/well at 3×10^6 cells/ml. Peripheral blood CD4+ T cells were isolated by depletion with CD8+ magnetic microbeads following the manufacturer's protocol (Miltteny Biotec) at week two or week three. Following depletion, the peripheral blood CD4+ T cells were stimulated with irradiated allogeneic whole peripheral blood mononuclear cells and TSST-1. Approximately one week later the cells were stimulated again and transfected twenty hours later.

PBMCs were resuspended at 2×10^7 cells/ml in complete media and 250 μ l of the cell suspension transfected with 25 μ g of a reporter construct and 0.25 μ g of a co-reporter expressing Renilla luciferase (pRLSV40; Promega) at 250 Volts and 960 microfarads using a Gene Pulser 11 (BioRad). Electroporated cells were plated, allowed to rest for 2 hours at 37°C, then activated with PMA (at a final concentration of 25ng/ml) and ionomycin (at a final concentration of 1.5 μ M; Sigma). Nine hours post-activation cells were harvested, washed twice in phosphate buffered saline, lysed in 50 μ l of reporter lysis buffer (Promega) and 20 μ l of each lysate was assayed for luminescence in an EG&G Berthold Lumat LB9507 luminometer according to the manufacturer's instructions using the Promega's Dual-Luciferase Reporter Assay System.

30

Analysis of CD40L Promoter Elements A series of 5' CD40L promoter deletions were PCR amplified and cloned into the firefly luciferase reporter plasmid pGL3-basic and the authenticity of all clones verified by DNA sequencing. Promoter activities for the 5' deletion constructs were compared to that of the full-length (-1860) CD40L promoter

following transfection into normal expanded T cells and activation with PMA and ionomycin.

Table 6 below, shows the promoter activity of various deletion mutants in activated T cells, some of which affect known transcription factor consensus sites including potential NF-AT and GATA-3 binding sites, as described by Shimadzu *et al.*, 1995.

Table 6. Activity of the CD40L promoter with 5' Deletions

5' Deletions	% Wild Type Activity
-1860	100%
to -1220	77%
to -951	91%
to -523	111%
to -280	47% +/- 12%
to -248	25% +/- 6.7
to -160	53%
to -87	45%
to -60	10% +/- 1.1%
to -26	4%

10

The results indicate that (1) deletion of CD40L promoter regions between -1860 and -523 had little or no effect on promoter activity; (2) deletion of the CD40L promoter to -427 resulted in slightly elevated promoter activity suggesting that the region may contain a negative regulatory element; and (3) deletion of the promoter to -280, further to -248, still further to -60 and still further to -26 reduces activity relative to the wild-type promoter suggesting the presence of activator sites between -427 and -280, between -280 and -248, between -87 and -60, and between -87 and -26.

15

A series of internal deletions were made within the context of the full-length -1860 promoter in pGL3-basic to address the possibility that deletion of large promoter regions may remove both positive and negative regulatory elements, and thereby result in cooperative effects. The various deleted CD40L promoter sequences were cloned into the promoter-less firefly luciferase reporter plasmid (pGL3-basic) and co-transfected into expanded T cells together with the SV40 driven Renilla luciferase control plasmid (pRLSV40). Firefly luciferase activity for each construct was normalized to Renilla luciferase activity and reported relative to that of the full-length promoter (-1860), with the length of each 5' deletion construct indicated relative to the transcriptional start site. All internal deletion clones were verified by DNA sequencing. The internal deletion promoter constructs were then transfected into expanded cultures of T cells and activities were compared to that of the -1860 promoter construct following activation with PMA and

20

25

ionomycin. The results presented as the mean \pm standard error of the mean, for a minimum of 2 independent transfections done in triplicate are provided in Table 7, below.

Table 7. Internal Deletions of CD40L and Promoter Activity

Deletion	% Wild Type Activity	Nucleotide Coordinates
-930 to -752	125%	941-1119
-730 to -524	77%	1141-1347
-503 to -428	71%	1368-1443
-406 to -301	36%	1465-1570
-320 to -291	34% \pm 3.5%	1551-1580
-300 to -281	50% \pm 18%	1571-1590
-280 to -231	37%	1591-1640
-230 to -211	15% \pm 3.7%	1641-1660
-230 to -110	13% \pm 2.2	1641-1761
-87 to -68	26% \pm 3%	1784-1803
-160 to -60	20% \pm 6.2%	1711-1811
-72 to -49	4% \pm 1.7%	1799-1822
-61 to -40	2.5% \pm 0.7%	1810-1834
-40 to +9	26%	1831-1880
+9 to +29	14% \pm 2.7	1880-1900

5

Internal deletions which resulted in reduced promoter activity include: (1) the -406 to -301 region, 3-fold reduction in activity relative to wild type; (2) the -320 to -291 region, 3-fold reduction in activity relative to wild type; (3) the -300 to -281 region, 2-fold reduction in activity relative to wild type; (4) the -280 to -231 region, 3-fold reduction in activity relative to wild type; (5) the -230 to -211 region, 6 to 7-fold reduction in activity relative to wild type; (6) the sequence immediately upstream of the -66 NF-AT site (deletion -87 to -68), 4-fold reduction in activity relative to wild type; (7) the -72 to -49 region, 25-fold reduction in activity relative to wild type; (8) the -61 to -40 region, 40-fold reduction in activity relative to wild type; and (9) the +9 to +29 region (downstream of the transcriptional start site), 14% reduction in activity relative to wild type.

10
15

In addition, various site-specific mutants constructed within the context of the full-length CD40L promoter (-1860 to +49) in pGL3-basic were co-transfected into normal expanded T cells together with the pRLSV40 control plasmid. Firefly luciferase activity for each construct was normalized to Renilla luciferase activity relative to that of the full-length wild-type promoter (Table 8). In the table, the positions of known transcription factor binding sites are indicated and numbered relative to the transcriptional start site

20

(+1) with data presented as the mean \pm standard error of the mean for a minimum of 2 independent transfections done in triplicate.

Table 8 Site Specific Mutations and Promoter Activity

Mutation	Wild Type Sequence	% Wild Type Activity
-1194 NFkB	GGGATTTCC	83%
-760 NF-AT	TTTTCC	91%
-599 NF-AT	GGAAAA	100% \pm 0%
-306	TTGTCACCTTC	24% \pm 4%
-269 GATA-3	GTGATA	67%
-264 NF-AT	GGAAAA	73% \pm 25%
-66 NF-AT	TTTTCC	32% \pm 4%
-37 to -29 TFIIB	GTGCGCT	53% \pm 19%
-30 to -25 TATA	CTTAAC	47% \pm 12%
-220 to -214	GGCAAG	26% \pm 3.5%
-214 to -208	AATGAA	31% \pm 6.9%
-208 to -202	TATATG	38% \pm 9.9%
-202 to -196	GAAGAA	36% \pm 4
-220 to -208	GGCAAGAATGAA	18% \pm 2.6
-72 to -66	AGCACA	49% \pm 48%
-66 to -60	TTTTCC	31% \pm 5.8
-60 to -54	AGGAAG	42% \pm 2
-54 to -48	TGTGGG	19% \pm 3.8
-48 to -42	CTGCAA	50% \pm 6%
-72 to -60	AGCACATTTTCC	10% \pm 1.8%
-66 to -54	TTTTCCAGGAAG	7% \pm 1.8%
-66 to -60 and -54 to -48	TTTTCC TGTGGG	14% \pm 2.2%
-66 to -60 and -48 to -42	TTTTCC CTGCAA	15%
-54 to -42	TGTGGCTGCAA	20% \pm 5.5%
-66 to -48	TTTTCCAGGAAGTGTGGG	11% \pm 1.5%
-72 to -60 and -54 to -48	AGCACATTTTCC TGTGGG	8% \pm 1.4%
-66 to -60 and -54 to -42	TTTTCC TGTGGGCTGCAA	5% \pm 15%

5

The results show that at least 4 regions of the CD40L promoter are critical to expression in activated T cells, as indicated by the levels of luciferase reporter expression and DNA footprinting studies. The regions of the CD40L promoter suggested by these results to be critical to expression in activated T cells include: (1) the site in the vicinity of nucleotide -306 (SEQ ID NO:12), the specific mutation of which results in a 4-fold down regulation of CD40L promoter activity; (2) the region between -230 and -196 (SEQ ID NO:14), based on deletion of the -230 to -211 region (SEQ ID NO:13), which resulted in an 6 to 7-fold downregulation of CD40L promoter activity, and site specific mutations of -220 to -214, -214 to -208, -208 to -202 or -202 to -197, which resulted in a 2.5 to 4-fold down regulation of promoter activity; and (3) the region between -77 and -40 (SEQ ID NO:15), based on the expression level of deletion mutants, wherein an internal deletion of -72 to -49 or -61 to -40 resulted in a 25-fold or 40-fold downregulation respectively. In addition, specific mutations in the composite AP-1/-66 NF-AT site together with a

previously unidentified site located between -48 and -54 indicates a contribution to transcriptional activation through the -48 to -54 site. (See Tables 7 and 8.)

It will be appreciated that some CD40L promoter regions may bind more than one transcription factor, and targeting a DNA-binding compound to the regulatory region of the CD40L promoter, described above, provides a means to inhibit CD40L promoter-mediated transcription through modulation of transcription factor-DNA interactions.

EXAMPLE 3

Hepatitis B (HBV)

A linearized unit-length HBV genomic fragment was prepared from an HBV plasmid containing 1.3 copies of a viral genomic sequence such that either the core, the preS1 or the X promoter was at the extreme 3' end. This fragment, when cloned into a reporter construct directionally, placed the promoter element immediately upstream of the reporter coding sequence in order to drive its expression. Luciferase reporter activities of these wild type core, X, and preS1 promoter constructs were evaluated by transient transfection experiments in cell lines of hepatic origin such as HepG2, Huh7, 22.1.5, and HepAD38. Subsequent mutant promoter constructs, prepared by site-directed mutations or linker scanner mutation, were prepared from these wild type clones using mutagenesis methods known in the art.

HBV Core Promoter

A luciferase reporter construct was constructed with a linearized full-length copy of the HBV genome, with the core promoter positioned immediately upstream and driving the expression of the reporter. Mutagenic primers containing blocks of 15 nucleotides of targeted sequence mutation were designed to generate a series of linker scanner mutant promoter reporter clones using either a *Morph*TM (5'Prime to 3'Prime, Boulder, CO) or a QuikChangeTM (Stratagene, La Jolla, CA) mutagenesis protocol.

Targeted segments of the promoter found to be resistant to mutagenesis were further sub-divided into smaller blocks of mutations consisting of 7-8 nucleotides. This series of linker scanner clones spanned the entire length of the core promoter segment. Mutagenic primers were also used to construct site-directed mutant constructs of known transcription factor binding sites including the hepatocyte nuclear factor sites, HNF3 and HNF4.

To determine potential critical regulatory elements in the core promoter, linker scanner analysis was performed using the series of systemic mutation clones constructed. Each linker scanner mutant construct was evaluated for promoter activity in

transient transfection experiments based on luciferase reporter activity in the hepatoma-derived cell lines HepG2 and HuH7. The HBV stably-transfected cell lines, 22.1.5 and HepAD38, were also used in the linker scanner analysis. An increase or decrease in relative luciferase reporter activity relative to the wild type indicated the presence of potential control elements critical to regulation of gene transcription.

Three regions of interest were identified in the linker scanning analysis. Mutations in domains 5, 8/9, and 13 resulted in 4-10 fold decrease in promoter activity (Table 9). All 3 regions align with cis-elements previously reported in the literature. Domain 5 contains sequences corresponding to a HNF4 transcription factor binding site (AGGACTCTTGGA SEQ ID NO:18). Domains 8/9 contain sequences corresponding to a HNF3 transcription factor binding site (proximal, HNF3-2, GACTGTTTGTTT, SEQ ID NO:17). Both of these protein factor sites have been described as important activation elements for the HBV core promoter. Domain 13 mutations abolish the TATA box sequence (CATAAA) of the promoter. A second HNF3 site (HNF3-1, domain 6) has been reported upstream of the one located in domains 8/9. However, mutation of this distal HNF3 site did not show any adverse effects in promoter activity.

Table 9 Reporter Analysis of Linker Scanner Mutation Clones of the HBV Core Promoter

Domain	Nucleotide Coordinates ¹	Linker Scanner Sequence	Wild Type Sequence	Percent Wild Type	
1	1601 – 1615	TACATGATATCTTCT	GCACGTCGCATGGAG	HepG2	HepAD38
2	1616 – 1630	CAAGAATTCCTATAA	ACCACCGTGAACGCC	88	147
3	1631 – 1645	ACAACCCGCGGTAA	CACCAAATATTGCC	79	65
4	1646 – 1660	CCTTGAGGCACGCG	AAGGTCTTACATAAG	28	38
5-1	1661 – 1668	CTCTAGAG	AGGACTCT	34	10
5-2	1668 – 1675	GGTCTAGA	TTGGACTC	22	18
6	1676 – 1690	GACGTCCGTGACCA	TCAGCAATGTCAACG	91	128
7	1691 – 1705	CAATCAAGATCTTAC	ACCGACCTTGAGGCA	76	93
8	1706 – 1720	GCAGGACCCTCGAG	TACTTCAAAGACTGT	7	9
9-1	1721 – 1728	GGTGCACC	TTGTTTAA	14	11
9-2	1728 – 1735	CTAGTGTT	AAGACTGG	24	17
10	1736 – 1750	TCTTCTAGATTTTCT	GAGGAGTTGGGGGAG	22	22
11	1751 – 1765	TCTCGGCTTGCCCA	GAGATTAGGTTAAAG	24	26
12-1	1766 – 1773	TGCGCATG	GTCTTTGT	103	103
12-2	1771 – 1780	GTGCACCTTC	TGTACTAGGA	37	36
13	1781 – 1795	TTAGTGCTTAAGCCC	GGCTGTAGGCATAAA	16	14
14	1796 – 1810	GCTCGAGTATACAAC	TTGGTCTGCGCACCA	37	68
15	1811 – 1825	TACAACGTACCCGG	GCACCATGCAACTTT	129	185
16	1826 – 1840	GGACAAGCTTAAGC	TTCACCTCTGCCTAA	229	247

The mutation of several additional regions, as shown in Table 10 showed a reduction in promoter activity of more than 4-fold. These regions, domain 5; domain 8/9 (HNF3 transcription factor binding site); and domain 13 (CATAAA box) appear to align

¹ HBV ayw strain

with the cis-elements (HNF-4/HNF-3, HNF-3/Sp1, and TATA box, respectively) reported in the literature, with the proximal HNF-3 site indicated as one critical element. The results of expression studies presented in Table 9 suggest that domain 8 (SEQ ID NO: 19); domain 8/9-1 (SEQ ID NO:20); and domain 13 (SEQ ID NO: 21) are involved in transcriptional activation.

Table 10 Reporter Analysis of Site-Directed Mutants of HNF3 and HNF4 Sites of the HBV Core Promoter

	Nucleotide Coordinates (HBV ayw Strain)	Site-Directed Mutant Sequence	Percent Wild Type HepAD38
Distal HNF3	1680 – 1691	CCAGGGCCCCGA	102
Proximal HNF3	1715 – 1726	GCCGCGGTCTGT	33
HNF4	1661 – 1672	CGTCCGCGGTGA	29

Following identification of the TATA box and the HNF4 and proximal HNF3 sites as the control elements most critical for core promoter activity, transcriptional activation as a result of the binding of the TATA binding protein (TBP) and the HNF transcription factors were further studied. It will be appreciated that failure of these protein factors to bind would result in down-regulation of the promoter.

Small DNA-binding compounds were utilized to test their ability to alter the transcription level from wild type and engineered HBV core promoters, either by interference and/or displacement of protein factor binding to its cognate nucleotide binding sequences, as further described in co-owned USSN 09/518,297, filed March 3, 2000. The results suggested that a compound binding site may be engineered into a promoter and thereby serve as a means for regulated gene expression of a coding sequence operably linked thereto.

preS1 promoter A luciferase reporter construct was generated containing a full-length copy of the HBV genome with the preS1 promoter positioned immediately upstream of the luciferase reporter gene. Using a wild type luciferase reporter clone, PreSpLuc, as a template, site-directed mutagenesis was performed using a *Morph*[™] (5'Prime→3'Prime, Boulder, CO) method to generate four mutants in known transcription factor binding sites and eight 15 bp linker scanner mutants. The mutagenized constructs were transiently transfected into Hep3AD38 and tested for promoter activity, as described above. Table 11 shows the results of the mutation analysis and the ability of the mutated promoters to drive luciferase expression.

Table 11. PreS1 Promoter Activity of Mutants

Construct	Coordinate	Mutated Sequence	% Wild type Activity
HNF1	2720-2732	5' TCGCGAACGGCAG	6
HNF3	2744-2755	5' ACAGCGCGCACA	40
Sp1	2765-2774	5' CGATATCTGC	48
TBP	2778-2784	5' GCGCGCC	34
Domain 1	2702-2716	5' GCGGCGAACTGCACG	182
Domain 2	2717-2731	5' AGCCGCGGGACGGCA	8
Domain 3	2732-2746	5' GGAACCCAGCTGACA	62
Domain 4	2747-2761	5' GCGCGCACACAGAGC	103
Domain 5	2762-2776	5' GTCTGCAGTTTGCGC	115
Domain 6	2777-2791	5' GCGCGCCTCTCTCC	34
Domain 7	2792-2806	5'CAGCTGACGCTATAA	53
Domain 8	2807-2821	5'GACGGGCCCTTTGAG	55

Among known transcription factor binding sites, the HNF1 site appears to be the most critical to preS1 promoter activity, as evidenced by the activity of the HNF1 mutant (16-fold reduction in activity). The domain 2 site (SEQ ID NO: 23) overlaps the HNF1 site and a domain 2 mutant showed a 13-fold reduction in activity. A domain 6 mutant showed a 3-fold reduction in activity suggesting that the domain 6 site (SEQ ID NO: 24) is also involved in transcriptional activation. Mutation of the HNF3, Sp1 and TBP binding sites resulted in a 2 to 3-fold reduction in reporter activity. In constructs with double mutations in HNF1 and TBP sites, there was no further reduction in reporter activity. In contrast, in Sp1 double mutants with either HNF3 or TBP, there was a further reduction relative to the reporter activity observed for the constructs with a mutation in HNF3, Sp1, or TBP alone.

To further map the HNF1 site, four serial 4 bp mutants with a 1 bp overlap were constructed and tested for promoter activity in luciferase reporter constructs (Table 12).

Table 12. HNF1 Linker-Scanning Mutagenesis

Construct	HNF1 sequence	% Wild type Activity
Wild type	GTTAATCATTACT	100
HNF1-A	TCGCATCATTAC	4
HNF1-B	GTTCCGAATTAC	3
HNF1-C	GTTAATACGGAC	4
HNF1-D	GTTAATCATGCAG	5

A series of point mutations spanning the HNF1 binding site were carried out and the mutants tested for luciferase expression following transient transfection. Four of seven mutants retained 14-42% of wild type activity as shown in Table 13, below.

Table 13. Mutants In The HNF1 Site Of The PreS1 Promoter

Consensus	GTT AAT NAT TAA C	Relative Luciferase Activity (%)		
Wild type	GTT AAT CAT TAC TT	100	100	100
mHNF1	TCG CAG ACG GCA GT	5	5	5
HNF1-4A	GTT GAT CAT TAC TT	-	5	-
HNF1-5A	GTT ACT CAT TAC TT	42	30	
HNF1-5B	GTT AGT CAT TAC TT	20	-	-
HNF1-6A	GTT AAG CAT TAC TT	-	6	-
HNF1-6B	GTT AAC CAT TAC TT	29	-	-
HNF1-9A	GTT AAT CAG TAC TT	-	3	-
HNF1-9B	GTT AAT CAC TAC TT	14	-	-
HNF1-5A6B	GTT ACC CAT TAC TT	-	-	9
HNF1-5A9B	GTT ACT CAC TAC TT	-	-	4

A fluorescence-based assay for characterization of ligands with DNA binding properties was carried out, the results of which are shown in Figure 2. A Hybridization Stabilization assay (HSA) was carried out using a 5'-fluorescent-labeled ssDNA and a 3'-Dabsyl labeled complementary strand of DNA. The oligonucleotides were designed to remain single stranded at room temperature until the ligand binds and duplexes the two strands resulting in quenching of the fluorescent signal. The direct binding of the ligand can then be unquenched by the presence of a more preferred sequence duplex. If a duplex does not have a preferential site for the particular ligand then the signal remains quenched. Figure 2 shows the results of a study where six different duplexes were tested against a particular ligand using a fluorescence-based assay for characterization of ligands with DNA binding properties. A Hybridization Stabilization assay (HSA) was carried out using a 5'-fluorescent-labeled ssDNA and a 3'-Dabsyl labeled complementary strand of DNA. The oligonucleotides were designed to remain single stranded at room temperature until the ligand binds and duplexes the two strands resulting in quenching of the fluorescent signal. The direct binding of the ligand can then be unquenched by the presence of a more preferred sequence duplex. If a duplex does not have a preferential site for the particular ligand then the signal remains quenched. Figure 2 shows the results of a study where six different duplexes were tested against a particular ligand. In the study, fluorescent and dabsyl labeled oligos at 25nM and 35nM were duplexed with the 21X ligand at 75nM. Various other duplexes were then added from 0 to 600nM to determine the sequence binding preference of the ligand. Reactions were in 225 μ l of

10mM HEPES pH 7.2, 50mM NaCl, 0.1mM EDTA and equilibrated at room temperature overnight.

The sequence that allows for the greatest fluorescence recovery is considered to be a preferred sequence for the ligand. The observed order of binding preferences for 21x was: HNF1-21X > TBP wild type > HNF1 wild type > HNF3 wild type > TBP mutant > HNF1 mutant (Figure 2). These results are consistent with the fact that in both the TBP and HNF1 mutant oligos, the majority of A/T bases were changed to G/C bases.

HBV X promoter

The HBV X promoter was analyzed by deletion and linker-scanning experiments similar to those described for the core promoter.

A luciferase reporter construct was constructed with a full-length copy of the HBV genome and the HBV X promoter (Fig. 3) positioned immediately upstream of a reporter coding sequence. Promoter constructs were prepared with successive blocks of 21 base pair mutated sequences from the HBV X promoter or known transcription factor binding sites.

Mutant constructs were transfected into the hepatoma-derived cell-line HepG2, HepG2 and cell lines stably transfected with HBV: 22.1.5 and HepAD38, and the expression of the luciferase reporter gene analyzed to determine HBV promoter activity. As indicated in Table 14, mutations in domains 3, 4 and 6 resulted in 28-51% of wild type activity when tested in 3 different cell lines.

Table 14. Linker Scanning Mutants of X Promoter

Construct	Coordinate	Mutated Sequence	% Wild Type Activity (HepG2)	% Wild Type Activity (2.2.15)	% Wild Type Activity (HepAD38)
Domain 1	1083-1103	5' CCTACTTCGCGACAGGGAGAT	110(343/103)	172/75	230/100
Domain 2	1104-1124	5' AACCAGGGCCCTTATGGGAGT	95/98	69	58
Domain 3	1125-1145	5' GTGCCCATCGCGAGTCCAAG	33/38	51	40
Domain 4	1146-1166	5' GCAAAATGGGATATCACCATT	59/36	51	45
Domain 5	1167-1187	5' AACTGCAGTGTAACCTGTGGG	113/105	83	119
Domain 6	1188-1208	5' TACAGATATCAAAAACAGTTA	33/40	28	33
Domain 7	1209-1229	5' GTTTTAGGATATCGTTTAACG	81/85	71	66
Domain 8	1230-1250	5' ACTATACGGATATCCCAAGGG	41/47	64	47
Domain 9	1251-1271	5' GATTACAAGAGATATCGAACG	48(56/39)	80/49	72/32
Domain 10	1272-1292	5' CAGTATTCCAGAAGATATCAG	51/50	62	70
Domain 11	1293-1313	5' GTGGGGAAGATATCACTTGAG	117/168	124	152
Domain 12	1314-1334	5' TTCTACCCACGGCGATATCAG	128	--	--
Domain 23	1335-1355	5' TCGCCAGAGTCGCGAAGCGA	102/100	110	85

The wild type sequence for domains 3 through 6, are as follows:

Domain 3: TGTAACAATACCTGAACCTT (SEQ ID NO:26)
 Domain 4: TACCCCGTTGCCCGGCAACGG (SEQ ID NO:27)
 Domain 6: GCTGACGCAACCCCCACTGGC (SEQ ID NO:28)

- 5 Two double mutants (domains 3 + 6 and domains 4 + 6), yielded a 7 to 9-fold reduction in activity relative to wild type controls when evaluated in the HepAD38 cell line (Table 15).

10 Table 15. Double Mutants of X Promoter

Mutant Clones	% Wild Type Activity
M3 + M4	43
M3 + M6	14
M4 + M6	11

- 15 Additional HBV-X promoter reporter constructs were made with mutations in various known transcription factor binding sites (Gustin K *et al.*, *Virology* 193, 653-660, 1993; Guo W *et al.*, *J. Virol.*, 1991; Nakamura I *et al.*, *Virology* 191, 533-540, 1992), and evaluated for luciferase reporter activity. The results of those studies which are presented in Table 16 suggest that the EF-C and E factor binding sites are important to activity of the HBV X promoter.

20 Table 16. Mutants of transcription factor binding sites of X Promoter

Domain	Coordinate	Mutated Sequence	% WT Activity (HepG2)	% WT Activity (2.2.15)	% WT Activity (Hep AD38)
NF1	1100-1119	CTCGCCAACCTTACAAGGCCT	109/109	119	93
2C	1119-1134	TTTCTGTGTAAACAAT	97/89	74	56
EF-C	1148-1168	CCCGTTGCCCGGCAACGGCC	46/44	36	28
E	1180-1202	CTGACGCAACCCCC	47/39	53	39
NF1	1209-1229	TGGGGCTTGGTCATGGGCCA	88/95	80	78
NF1	1216-1236	TGGTCATGGGCCATCAGCGC	74/77	110	71
X-PBP	1229-1245	ATCAGCGCATGCGTGGA	56/61	69	48

- 25 Given that all HBV-X promoter reporter constructs contained the entire HBV genome, two additional constructs were made: X enhancer/promoter reporter (XpLuc200, Table 17), and the entire HBV genome without X enhancer/promoter-reporter Xp(-) Luc3000, Table 17), to exclude the possibility that there is read-through from other HBV promoters. The XpLuc200 construct was made by amplifying a clone from each of domain 3, 4, and 18 with the forward and reverse primers (SEQ ID NO:29)

and (SEQ ID NO:30), respectively, followed by cloning into the pGL3 Basic vector. The "Xp(-) Luc3000" construct was made by subjecting the wild type construct XpLuc (29-1-5) to site-directed mutagenesis by *Morph*TM method. All "XpLuc200" constructs, 3.6, 4.9, 18.13, and 29-1-5, showed approximately 1.5 to 2 fold promoter activity relative to that of each full-length construct, while the Xp(-) Luc3000 construct (29-1-5(-Xp)) showed no promoter activity. These results support the conclusion that the reporter activity presented in Tables 14 and 16 reflect an effect on the HBV X promoter alone, and is not due to upstream HBV promoters (Sp, preSp or Cp).

Table 17. XpLuc200 and Xp(-)Luc3000 Constructs

Construct	Presence of promoters				Proximal promoter of reporter gene
	Cp	PreS1p	Sp	Enh1/Xp	
XpLuc200	-	-	-	+	Xp
Xp(-)Luc3000	+	+	+	-	Sp

EXAMPLE 4

Vancomycin-Resistant Enterococci (VRE)

A modified pAM401 plasmid (ATCC) was designed containing a VRE promoter sequence upstream of the luciferase gene in a background allowing for growth and maintenance in *E. coli* and *Enterococcus* species. The *vanH* promoter (SEQ ID NO:31) was PCR amplified from VRE strain CSUC4 with *NcoI* and *Sall* sites added to it. The pAM401 plasmid was cut using *XbaI* and *Sall* and triple ligated to the firefly luciferase gene isolated from pGL3 basic (Promega) by cutting *XbaI* to *NcoI* and incorporating the *vanH* promoter into the construct.

Transformants were screened by restriction analysis following PCR amplification and the resulting plasmids electroporated into L-threonine treated *Enterococcus* strain CSUC-4.

Site directed mutagenesis of the VRE promoter region was carried out by systematically altering short 8 to 10 bp regions of the consensus promoter sequence, including the -35 consensus binding site within the phosphorylated VanR footprint (Arthur *et al.*, 1992).

20 linker scanning mutants designated M2-M21 were generated and nucleic acid constructs containing the VRE promoter sequence upstream of the luciferase gene were subcloned into the a pRLUC parent vector and transformed into *E. coli*. Figure 7 presents the sequences of *vanH* promoter mutants M2-M21, wherein each group of 10 nucleotides

in the original vanH promoter sequence shown in the figure was replaced with the mutant sequence, e.g., in M2 the CCCGGGGGGC sequence was inserted in place of the wild type TAATTTTTA sequence. The position of the mutations and corresponding luciferase activity is shown in Table 18.

5 The luciferase expression of selected promoter mutants was analyzed in 3 clinical strains of *Enterococcus* to ascertain if the effect of modified promoter elements on expression is consistent between strains. CSUC-4, the initial strain assayed, UCD-3 and UL-178 were used in the analysis. The M9 clone consistently resulted in the highest luciferase activity of the mutants tested. Among the other mutants M8 also had a
10 consistent effect on induction.
(See Table 18.)

Table 18. vanH Promoter Mutants And Reporter Activity

Construct	Coordinate	Mutated Sequence	% Wild Type Activity (UCD3)	% Wild Type Activity (UL17)	% Wild Type Activity (CSUC4)
M2	-100 to -91	CCCGGGGGGC	120.4	53.6	10.7
M3	-90 to -81	TTCCCCGGGA	108.7	38.7	10.3
M4	-80 to -71	CCTAGGCGAG	—	—	0.4
M5	-70 to -61	GGCGCGCGGA	—	—	1.6
M6	-60 to -51	GCGCGCCCGG	36.5	10.3	0.4
M7	-50 to -41	CCACGCGCGC	45.5	18.9	1.8
M8	-40 to -31	GCGCGCTCCC	0.1	0.0	1.3
M9	-30 to -21	ATTGGTACCA	152.5	100.9	1202
M10	-20 to -11	GGCGCGCTGC	—	—	32.6
M11	-10 to -1	TCAGCGCGCA	1.3	—	1405
M12	+1 to +10	ATGCGCGCAT	—	—	1737
M13	+11 to +20	TTAACGGGGA	—	—	770.7
M14	+21 to +30	TGGAGCGCGC	—	—	115.2
M15	+31 to +40	TCCGCGCGCT	—	—	50.6
M16	+41 to +50	CACGCGCGCA	—	—	23.6
M17	+51 to +60	ACGGAATTCA	—	—	2.4
M18	+61 to +70	AAAGCGCGCG	—	—	76.3
M19	+71 to +80	GGTACCAAGG	—	—	57.3
M20	+81 to +90	GACAGCTGCT	—	—	0.0
M21	+91 to +100	TTGGTTAACG	—	—	12.6

15 Each linker scanner promoter mutant was tested for activity, with both up- and down-regulation observed. The largest decrease was to 0% of wild type activity and the largest increase was 1737% of wild type activity. All mutants in the region reported to be footprinted by phosphorylated VanR (M2-M8) showed decreased activity. Increased activity was observed in mutants spanning nucleotides -30 to +20 (M9-M13), suggesting
20 the possibility of a repressor binding site in this region. Of particular interest are putative

activator sequences which correspond to M6 (SEQ ID NO:32) and M8 (SEQ ID NO:33), and a putative repressor sequence which corresponds to M12 (SEQ ID NO:34).

EXAMPLE 5

5 Androgen receptor

The androgen receptor promoter from nucleotides -6000 to +1100 (Figs. 8A-C, SEQ ID NO:35), was cloned from genomic DNA by PCR using GenBank sequences and subcloned into pGL3 basic (Promega) for subsequent transient transfection.

10 A large series of deletion constructs were made and tested in the androgen dependent prostate cell line, LNCaP, following transient transfection.

The deletion constructs were made using the following PCR primer pairs: for the -6000+1 construct (SEQ ID NO:36) and (SEQ ID NO:37); for the -4000+1 construct (SEQ ID NO:38) and (SEQ ID NO:39); for the -2000+1 construct (SEQ ID NO:40) and (SEQ ID NO:41); for the -2000+1100 construct (SEQ ID NO:42) and (SEQ ID NO:43); for the -200+1 construct (SEQ ID NO:44) and (SEQ ID NO:45); for the -200+100 construct (SEQ ID NO:46) and (SEQ ID NO:47); for the -400+1 construct (SEQ ID NO:48) and (SEQ ID NO:49); for the -300+1 construct (SEQ ID NO:50) and (SEQ ID NO:51); for the -150+1 construct (SEQ ID NO:52) and (SEQ ID NO:53); for the -100+1 construct (SEQ ID NO:54) and (SEQ ID NO:55); for the -50+1 construct (SEQ ID NO:56) and (SEQ ID NO:57); for the -200+125 construct (SEQ ID NO:58) and (SEQ ID NO:59); for the -200+71 construct (SEQ ID NO:60) and (SEQ ID NO:61); and for the -200+50 construct (SEQ ID NO:62) and (SEQ ID NO:63).

The following deletion constructs were tested for luciferase activity with the results in parentheses presented as % of the -200+1 control: -6000+1 (38%), -4000+1 (31%), -2000+1 (45%), -400+1 (93%), -300+1 (100%), -200+1 (100%), -150+1 (109%), -100+1 (62%), -50+1 (28%), -2000+1100 (100%), -200+1100 (459%), +1+1100 (114%), -200+200 (562%), -200+150 (474%), -200+125 (314%), -200+100 (168%), -200+71 (153%) -200+50 (87%) and basic promoter construct (5%).

30 The results of transient transfection assays in the AR+ cell line LNCaP illustrate a repressor, and multiple activator sites at nucleotides -150 to -100 (homopurine stretch), -100 to -50 (SP1 site), and -50 to +1 (helix loop helix binding site).

35 The results indicate that: (1) the untranslated region (UTR) from +1 to +1100 contains two critical regions for optimal activity, sites between +125 and +100 and between +71 and +50; (2) a repressor site may exist between -2000 and -400; and (3) the activity of the proximal promoter region is derived from sequences between -150 and -100

(approximately 2-fold) and between -100 and -50 (an additional 2-3 fold) and -50 to +1 (an additional 4-5 fold).

Additional site specific mutants were generated as follows: delta HP, a 40 bp internal deletion of the homopurine stretch, delta HP (5'), delta HP (3'), HLH-us, SPI, HLH-ds, the 3' 10 bp of HLH-ds (HLH-3), the 5' 10 bp of HLH-ds (HLH-5) and a double mutant of delta HP and HLH-ds (delta HP /HLH-ds) all in the context of the 200+1 construct. The results of transient transfection studies in LNCaP cells expressed as % of the -200+1 control are presented in Table 19.

Table 19. Luciferase activity of Promoter constructs

Promoter Construct	Luciferase Expression (% of control)
-200+1 (control)	100
delta HP	40
delta HP (5')	41
delta HP (3')	89
HLH-ds	63
delta HP/HLH-ds	40
HLH-us	88
SP1	111
HLH-3	42
HLH-5	136

These results suggest that: (1) the 5' portion of the homopurine region represents all of the activity from -150 to -100; (2) the region 3' of the downstream helix-loop-helix sequence contains another 2-fold of activity; and (3) the downstream helix-turn-helix site contains 1.5-fold activity.

Of particular interest are the HLH-ds and HLH-3 deletion mutants and the 5' HP mutant, which resulted in a significant decrease in luciferase activity indicating the presence of an activator site. The corresponding wild type sequences for these mutants are presented as SEQ ID NO: 64, SEQ ID NO:65 and SEQ ID NO:66, respectively.

EXAMPLE 6

Her2

A 2000-bp fragment of the human Her2 promoter (Fig. 9, SEQ ID NO:67) was PCR amplified from genomic DNA using the following oligonucleotides:

5'- GCA CGC GTA AGC TTC AGG CCC CAC AAA ACC TA-3' (SEQ ID NO:68) and
5'- CGC TCG AGC CAT GGC TCC GGC TGG ACC CGG CTG GG-3' (SEQ ID NO:69).

This purified fragment was subcloned into the vector pGL3-basic (Promega) at the

NcoI and HindIII sites for use in transient transfection assays in breast carcinoma cell lines MCF-7 (low HER2 expression) and MDA-MB-453 (high HER2 expression).

In addition, several deletion constructs were made in a Her2 luciferase reporter containing a 2 kb promoter fragment cloned into pGL3-Basic. The reporters were transiently transfected into the MCF7 and ZR75 cell lines. Table 20 shows the reporter activity for each promoter construct with the modified sequence portion indicated as underlined. The results indicate that the critical regulatory sites for the Her2 promoter lie between nucleotides -125 and -50.

Table 20. Luciferase Reporter Activity of Various Her2 Promoter Constructs in MCF7 and ZR75 Cells.

Construct	Sequence (modification presented as underlined)	% Wild Type Activity (MCF7/ZR75)
Her2 wild type	GAGCTGGGAGCGCGCTTGCTCCCAATCACC GGAGAAGGA	100/100
100 to 85	GATGGATCCTATATACCGCTCCCAATCACC GGAGAAGGA	22/33
80 to 65	GAGCTGGGAGCGCGCTTGCTCCAGGATCCATT CACCTGA	30/29
90 to 75	GAGCTGGGAGCGATGGATCCAAACCGAACC GGAGAAGGA	9/12
87 to 79	GAGCTGGGAGCGCGCGGATCCCAATATCACC GGAGAAGGA	16/12
84 to 76	GAGCTGGGAGCGCGCTTGAGGATCCGAACCG GGAGAAGGA	18/23
84 to 78	GAGCTGGGAGCGCGCTTTAGATCTATCACC GGAGAAGGA	1/17
81 to 76	GAGCTGGGAGCGCGCTAAGCTTCAATCACC GGAGAAGGA	1/23
90 to 82	GAGCTGGGAGCAATGGATCCACCAATCACC GGAGAAGGA	505/434
84 to 81	GAGCTGGGAGCGCGCTTTAGACCAATCACC GGAGAAGGA	306/297
93 to 85	GAGCTGGGATAGGATCCTCTCCCAATCACC GGAGAAGGA	41/62
81 to 73	GAGCTGGGAGCGCGCTTGCTCAAGGATCCAGAGGAAGGA	70/71
93 to 88	GAGCGGATCCCGCGCTTGCTCCCAATCACC GGAGAAGGA	1/46
87 to 82	GAGCTGGGAGGGATCCTGCTCCCAATCACC GGAGAAGGA	1/72
75 to 70	GAGCTGGGAGCGCGCTTGCTCCAAGCTTCC GGAGAAGGA	1/132
75 to 70	GAGCTGGGAGCGCGCTTGCTCCGGATCCCC GGAGAAGGA	60/60

To further delineate the critical site(s), a series of linker scanner Her2 luciferase reporter mutants was made from nucleotides -130 through -55. The constructs designated 10085, 9075, 8065, and 7055 (indicating the bases mutated; e.g., 10085 indicates that bases from -100 through -85 were mutated, etc.) were tested in transient transfections in ZR75 and MCF7 cells with the results presented in Table 23 as % activity relative to the wild type promoter.

The results clearly implicate the -90 to -75 region as critical to the activity of the Her2 promoter.

Mutations were made in various regions of the Her2 promoter, including an AT-rich region around and including a putative TATA box (TB, "TATAAGA"), a putative TATA box (T5B, CTTGAGGAAGGATCCGAATGAAGTTGT), an AT stretch downstream of the putative TATA box (T3B, CTTGAGGAAGTATAATCCGGAAGTTGT), a putative ets site (EP), a double mutant of the AT-rich region around and including the putative TATA box

(TATA/Ets, CTTTCGATCGGATCCGCCGGAAGTTGT), and the putative ets site (TBEP, "GAGGAA") as well as a deletion to -215. Sequence modifications are indicated as underlined.

5 Luciferase reporter constructs were prepared with the various Her2 promoter sequences immediately upstream of the reporter coding sequence. The reporters were transiently transfected into MCF7 and ZR75 cells and the resulting luciferase expression reported as the % of wild type (Table 21).

10 Table 21. Luciferase Reporter Activity of Various Her2 Promoter Constructs

Promoter Construct	% WT Activity MCF7 cells	% WT Activity ZR75 cells
Basic	3.3	2.1
Wild type	100	100
-215	143	195
TB	796	432
T5B(TATA)	64	44
T3B	521	351
EP (ets)	69	62
TBEP	843	449

15 The data suggest that sequences upstream of nucleotide -215 are not critical for regulation. As shown in Table 21, mutating the TATA box or the ets site causes a modest decrease in transcription, suggesting that a repressor site lies just downstream of the TATA box. The sequence near the putative TATA box and putative ets site is shown below.

CTGCTTGAGGAAGTATAAGAATGAAGTTGT
 ets TATA box

20 An additional deletion construct, -50, was made in a Her2 reporter containing a 2 kb promoter fragment cloned into pGL3-Basic and compared to the -215 deletion. The reporters were transiently transfected into MCF7 and ZR75 cells line. The results indicate that critical regulatory sites for the Her2 promoter lie in the -215 to -50 region.

25 Several additional deletion constructs were made in a Her2 luciferase reporter containing a 2 kb promoter fragment cloned into pGL3-Basic. The reporters were transiently transfected into MCF7 and ZR75 cells line. The results presented in Table 22 (expressed as % wild type luciferase activity), indicate that the region of the Her2 promoter between -125 and -50 contains critical regulatory sites.

30

Table 22. Luciferase Reporter Activity of HER2 Deletion Constructs

Her2 promoter construct	MCF7 cells	ZR75 cells
Basic	6.1	4.1
Wild type	100	100
deletion of 5' end to -215	215	167
deletion of 5' end to -150	58	41
deletion of 5' end to -125	65	40
deletion of 5' end to -100	27	16
deletion of 5' end to -50	4.8	3.7

Further experiments were carried out to determine if the sequences identified as important in ZR-75 and MCF-7 cells are also important in other breast cancer cell lines. Two cell lines SKBR-3 (SK) and BT-474 (BT), were selected which overexpress Her2 at higher levels than do either ZR-75 or MCF-7 cells. A summary of the data from 3 transient transfections is presented below in Table 23, together with the results of parallel studies done in ZR-75 cells (ZR).

Table 23. Luciferase Reporter Activity of Her2 Mutants in BT-474 and SKBR-3 Cells

Reporter	Transfection 1			Transfection 2		Transfection 3		
	SK	BT	ZR	SK	BT	SK	BT	ZR
Her2 WT	100	100	100	100	100	100	100	100
Basic	0.8	3.2	2.0	0.6	2.8	0.5	3.5	2.1
9082-1	150	293	416	269	243	177	296	337
8481-3	295	286	406	159	220	197	251	305
TATAB-3	446	1014	881	733	961	500	718	608
8478-10	49	40	19.5	41	52	23	52	27
CATb-21	27	69	52	25	76	20	72	59
9075-8		10	30	10	9	39		
-50-3	3	6	4	2	7.5			
-100-3	10	21	21	9	25			
-150-10	19	45	48	16	51			

The comparative results of luciferase expression assays in the 3 cell lines suggest the following: (1) the Her2 promoter is 4-5 fold stronger in SKBR-3 cells than in BT-474 cells and 3-4 fold stronger than in ZR75-1 cells; (2) the TATA-Bam mutation results in less up-regulation in SKBR-3 cells than in the other two cell lines; (3) the CCAAT box is more important in SKBR-3 (4-5 fold decrease) than in either ZR75-1 (2-fold down) or BT-474 (less than 2-fold down) cells and (4) the CCAAT box may be an appropriate target for regulation of Her2.

Based on the results provided above, sequences of interest for regulated expression of Her2 are a repressor sequence "GAATGAAGTT" (SEQ ID NO:70) downstream of the putative TATA box at -23 to -19; the complex regulatory region "CGCTTGCTCCCAATC" (SEQ ID NO:71), which has both activator and repressor components and the TATA box/ets site, "GAGGAAGGTATAA" (SEQ ID NO:72), wherein the ets sequence is "GAGGAAG" and the TATA box sequence is "TATAA".

EXAMPLE 7

10 Beta-lactamase (Bla) promoter

The natural beta-lactamase promoter P3 (SEQ ID NO:73), shown below, lies near the coding sequence of beta-lactamase (bla), initiating transcription at 35 bases 5' to the ATG translation initiation codon. The P3 promoter contains a Pribnow box (GACAATA) at the -10 region and a -35 consensus sequence, TTCAAA. The -35, -10, start site and ribosome binding site, respectively in the 5' to 3' order, are indicated as underlined, below.

20 GACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTT
CTAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATA
ATA TTGAAAAAGGAAGAGT (natural beta-lactamase promoter P3, SEQ ID NO:73)

Renilla luciferase reporter constructs were prepared in the pACYC177 vector, wherein the wild type beta-lactamase promoter driven Renilla luciferase construct was designated pBla-rluc. The control promoter-less construct was designated pNull-rluc, and the luciferase negative construct designated pBla-bla.

25 Bla promoter mutants (designated "M#") of the natural P3 bla promoter were generated by systematically altering the base pairs of the entire bla promoter sequence (from nucleotides -101 to +43). In general, mutants were generated by introducing 6 to 12 base pair mutations at different locations of entire Bla promoter by Quick Change, by replacing purines with pyrimidines and vice versa and incorporating restriction sites in the sequence.

30 Luciferase activities of various Bla mutants were measured in lysates prepared from *E. coli* XL1 Blue replicates and compared to that of wild type pBla-rluc. Mutants which exhibited significantly decreased luciferase activity include the -35 region (-41 to -30, M6); the -10 region (-17 to -6, M8); the start site (-5 to +7, M9); and +20 to +31 (M11) which exhibited luciferase activities which were reduced to 24%, 29%, 15% and 2% of wild type, respectively, as shown in Table 24, below.

Table 24. Sequences of Bla Promoter Mutants and Luciferase Reporter Activity

Mutant	Location	Wild Type Sequence	Mutated Sequence	Luciferase Activity (% Wild Type)
M6	-41 to -30	AATACATTCAAA	CCGGCCGGACCC	24%
M21	-35 to -30	TTCAAA	GGACCC	28%
M8	-17 to -6	CATGAGACAATA	ACGCGTCACCGC	29%
M30	-8 to -3	TAACC	CGCCAA	24%
M9	-5 to +7	ACCCTGATAAAT	CAAAGTCGACCG	15%
M11	+20 to +31	TTGAAAAAGGAA	GGGCCCCCTTCC	2%

Luciferase activity of mutants M6 (-41 to -30; SEQ ID NO:75) and M21 (-35 to -30) revealed that the -35 region is critical to promoter activity, as indicated by a reduction in luciferase activity to 24% and 28%, respectively. Luciferase activity of mutant M8 (-17 to -6; SEQ ID NO:76) and M30 (-8 to -3) revealed that the -10 pribnow box region is also critical, as indicated by a reduction in luciferase activity to 29% or 24%. Two additional regions important for luciferase activity are the start region (M9; SEQ ID NO:77) and the ribosome binding site region (M11; SEQ ID NO:78), as indicated by a reduction in luciferase activity to 15% and 2%, respectively.

The sequence of the -101 to +35 region of a modified BlaMT promoter (SEQ ID NO:74) is presented below with lower case letters indicating mutations relative to the natural P3 Bla promoter sequence.

GACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATcTGTTTgTTCTTc
 TAgAcACATTCAcAcATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATgA
 cATTGAgAAAGGAAGAGT (modified BlaMT promoter, SEQ ID NO:74)

Table 25. Sequences of pBlaMT and Mutant pBlaMT Constructs

Mutants	Sequence(-35 to +7 of BlaMT promoter)
PblaMT	<u>TT</u> CACACATGTATCCGCTCATGAGACAATAACCCTGATAAAT
pBlaMT(-35)	<u>TT</u> tAaA <u>t</u> ATGTATCCGCTCATGAGACAATAACCCTGATAAAT
pBlaMT(-10)	<u>TT</u> CACACATGTATCCGCTCATGAGAtAATAA <u>tt</u> CTGATAAAT
pBlaMT(-10p)	<u>TT</u> CACACATGTATCCGCTCATGAGACAATAACCCTGATgAAT
pBlaMT(-10/+)	<u>TT</u> CACACATGTATCCGCTCATGAGACAATAA <u>ttt</u> TGAcgAAT
pBlaMT (+1)	<u>TT</u> CACACATGTATCCGCTCATGAGACAATAAC <u>tt</u> TATAAAT

Table 25 depicts the location and sequence of various mutant BlaMT promoter constructs. Lower case letters indicate the mutations relative to the pBlaMT sequence and underlined sequences indicate the location of potential compound binding sites. As detailed in co-owned PCT Publication No. WO 00/52179, when a sequence immediately downstream of the start site in various pBlaMT mutant constructs [e.g.,

pBlaMT, pBlaMT(+1), pBlaMT(-35) and pBlaMT(-10)] was targeted by a DNA binding compound, the activity of each promoter was up-regulated.

5 The data presented herein provides an analysis of the regulatory regions of various promoters and shows that once the regulatory region of a promoter is identified, it can be targeted by both cellular factors (native or exogenously provided) and by compounds in order to effect regulated expression of a coding sequence operably linked thereto.

Table 26. Sequence Listing Table

DESCRIPTION	SEQ ID NO
cyclin D1 promoter -1745 to +155 (wild-type, Fig. 4)	1
cyclin D1 primer for PCR amplification of promoter from genomic DNA 5'-GCA CGC GTG CTA GCC AGC TGG GCC GCC CTT GT-3'	2
cyclin D1 primer for PCR amplification of promoter from genomic DNA 5'-ATC CAT GGA AGC TTT GGG GCT CTT CCT GGG CA-3'	3
cyclin D1 primer for PCR cloning of 5' deletions: 5'-GCA CGC GTG CTA GCT GGA GCC TCC AGA GGG CTG T-3'	4
cyclin D1 -30-21 wild type sequence: GAGTTTGT	5
cyclin D1 -30-24 wild type sequence: GAGTTT	6
cyclin D1 CRE wild type sequence: CAGTAACGTACACGG	7
cyclin D1 (+1 to +9) wild type sequence: CCTCCAGAGG	8
CD40L promoter (human, full-length) -1860 to +49 (Fig. 5A-C)	9
CD40L, D1 primer for PCR amplification of promoter from genomic DNA 5'-TTA TGA TAC CTC GAG GGG AGA GCA TTC AGG AAG ATG-3'	10
CD40L, D1 primer for PCR amplification of promoter from genomic DNA 5'-TGA ATC ACG AAG CTT TGG TAT CTT CTG GCA GAG AAG-3'	11
CD40L site between -320 and -297: GAT GAA TTT GTC ACT TTC CTT GAA	12
CD40L site between -230 and -211: GAC ATT TCA AGG CAA GAA TG	13
CD40L site between -230 and -196: ACA TTT CAA GGC AAG AAT GAA TAT ATG GAA GAA GA	14
CD40L site between -77 and -40: TACGA AGCACATTTTCCAGGAAGTGTGGGCTGCAACG	15
HBV core promoter sequence (Fig. 1A)	16
HBV core promoter proximal, HNF3-2 site: GACTGTTTGT	17
HBV core promoter HNF4 transcription factor binding site: AGGACTCTTGA	18
HBV core promoter domain 8 wild type sequence: TACTTCAAAGACTGT	19
HBV core promoter domain 8 and 9-1 wild type sequence: TACTTCAAAGACTGTTTGTAA	20
HBV core promoter domain 13 wild type sequence: GGCTGTAGGCATAAA	21
HBV pre-S1 promoter sequence (Fig. 1B)	22
HBV pre-S1 promoter domain 2 wild type sequence: CTA GTT AAT CAT TAC	23
HBV pre-S1 promoter domain 6 wild type sequence: TTA TAT AAG AGA GAA	24
HBV-X promoter sequence (Fig. 3)	25
HBV-X promoter domain 3 wild type sequence: TGTAACAATACCTGAACCTT	26
HBV-X promoter domain 6 wild type sequence GCTGACGCAACCCCACTGGC	28
Forward primer for construction of XpLuc200 CACCGAAGCTTAAGCAGGCTTTCACCTTCTCG	29
Reverse primer for construction of XpLuc200 CAGTACCGGAATGCCAAGCTTCGATG	30

DESCRIPTION	SEQ ID NO
vanH promoter sequence (Fig. 6) GAGATGTATATAATTTTTAGGAAAATCTCAAGGTTATCTTTACTTTTTCTTAGG AAATTAACAATTTAATATTAAGAAACGGCTCGTTCTTACACGGTAGACTTAATAC CGTAAGAACGAGCCGTTTTCGTTCTTCAGAGAAAGATTTGACAAGATTACCATT GGCATCCCCGTTTTATTTGGTGCCTTTCACAGAAAGGGTTGGTCTTAATT	31
vanH wild type promoter sequence corresponding to M6: TTAGGAAATT	32
vanH wild type promoter sequence corresponding to M8: TATTAAGAAA	33
vanH wild type promoter sequence corresponding to M12: CGTAAGAACG	34
Androgen receptor (AR) promoter sequence from -6000 to +1100 (Figs. 8A-C)	35
AR: forward PCR primer for construction of -6000+1 deletion construct CACGCGTGGTACCTCTAGAAAATAATTCCCAATATTGAATCCC	36
AR: reverse PCR primer for construction of -6000+1 deletion construct AGCTGGCTCCCCGGGATCTCGGAGGGGGCGC	37
AR: forward PCR primer for construction of -4000+1 deletion construct CACGCGTGGTACCAGACAGTGACAGGACTTAAACGGGGAAAT	38
AR: reverse PCR primer for construction of -4000+1 deletion construct AGCTGGCTCCCCGGGA	39
AR: forward PCR primer for construction of -2000+1 deletion construct CACGCGTGGTACCTATACACATTATGTCTTTTAAATGAC	40
AR: reverse PCR primer for construction of -2000+1 deletion construct AGCTGGCTCCCCGGGATCTCGGAGGGGGCGC	41
AR: forward PCR primer for construction of -2000+1100 deletion construct CACGCGTGGTACCTATACACATTATGTCTTTTAAATGAC	42
AR: reverse PCR primer for construction of -2000+1100 deletion construct CCGCCATGGTGAGCTTGGCTGAATCTTCCA	43
AR: forward PCR primer for construction of -200+1 deletion construct CCGGGTACCTGCCCTCGCCACGCTGCGCC	44
AR: reverse PCR primer for construction of -200+1 deletion construct AGCTGGCTCCCCGGGATCTCGGAGGGGGCGC	45
AR: forward PCR primer for construction of -200+100 deletion construct CCGGGTACCTGCCCTCGCCACGCTGCGCC	46
AR: reverse PCR primer for construction of -200+100 deletion construct AGCTGGCTCCCCGGGATCTCGGAGGGGGCGC	47
AR: forward PCR primer for construction of -400+1 deletion construct: CAGAACATTTCTCTATCGATAGGTACCGAGCAGGTATTCCTATCGTCCTTTTCC	48
AR: reverse PCR primer for construction of -400+1 deletion construct: GGAAAAGGACGATAGGAATACCTGCTCGGTACCTATCGATAGAGAAATGTTCT G	49
AR: forward PCR primer for construction of -300+1 deletion construct: CAGAACATTTCTCTATCGATAGGTACCAATCTGGAGCCCTGGCGCCTAAACCT	50
AR: reverse PCR primer for construction of -300+1 deletion construct: AGGTTTAGGCGCCAGGGCTCCAGATTTGGTACCTATCGATAGAGAAATGTTCT G	51
AR: forward PCR primer for construction of -150+1 deletion construct: CAGAACATTTCTCTATCGATAGGTACCGCGTTAGCGCGCGGTGAGGGGAG	52

DESCRIPTION	SEQ ID NO
AR: forward PCR primer for construction of -100+1 deletion construct CAGAACATTTCTCTATCGATAGGTACCGGGAAAAGGAGGTGGGAAGGCAAGGAGGCC	
AR: reverse PCR primer for construction of -100+1 deletion construct GGCCTCCTTGCCTTCCCACCTCCTTTTCCCGGTACCTATCGATAGAGAAATGTTCTG	55
AR: forward PCR primer for construction of -50+1 deletion construct CAGAACATTTCTCTATCGATAGGTACCCTCGCAAACCTGTTGCATTTGCTCTCCA CCTCCC	56
AR: reverse PCR primer for construction of -50+1 deletion construct GGGAGGTGGAGAGCAAATGCAACAGTTTGCGAGGGTACCTATCGATAGAGAAATGTTCTG	57
AR: forward PCR primer for construction of -200+125 deletion construct CCAGTGCTGTACAGGAGCCGAAGGGACGCACCCCATGGAAGACGCCAAAAACATAAAGAAAGGCC	58
AR: reverse PCR primer for construction of -200+125 deletion construct CCTTTCTTTATGTTTTTGGCGTCTTCCATGGGGTGCGTCCCTTCGGCTCCTGTA CAGCACTGG	59
AR: forward PCR primer for construction of -200+71 deletion construct CCACAGGCAGAGGAGGCGACAGAGGGCCATGGAAGACGCCAAAAACATAAAGAAAGGCC	60
AR: reverse PCR primer for construction of -200+71 deletion construct CCTTTCTTTATGTTTTTGGCGTCTTCCATGGCCCTCTGTGCGCTCCTCTGCCTGTGG	61
AR: forward PCR primer for construction of -200+50 deletion construct GGGAGAGCGGGACGGTCCGGAGCAAGCCCACCATGGAAGACGCCAAAAACA TAAAGAAAGGCC	62
AR: reverse PCR primer for construction of -200+50 deletion construct GGCCTTTCTTTATGTTTTTGGCGTCTTCCATGGTGGGCTTGCTCCGGACCGTC CCGCTCTCCC	63
AR wild type HLH-ds sequence: TGTTGCATTTGCTCTCC	64
AR wild type HLH-3 sequence: GCTCTCCACCTCCCAG	65
AR wild type 5' HP sequence: GGTGAGGGGAGGGGAGAAAAGGAAA	66
Her2 promoter sequence (Fig. 9)	67
PCR primer for amplification of a 2000-bp fragment of the human Her2 promoter 5'-GCA CGC GTA AGC TTC AGG CCC CAC AAA ACC TA-3'	68
PCR primer for amplification of a 2000-bp fragment of the human Her2 promoter 5'-CGC TCG AGC CAT GGC TCC GGC TGG ACC CGG CTG GG-3'	69
Her2 wild type repressor sequence downstream of the putative TATA box: GAATGAAGTT	70
Her2 wild type complex regulatory region: CGCTTGCTCCCAATC	71
Her2 wild type TATA box/ets site: GAGGAAGGTATAA	72
natural beta-lactamase promoter P3 GACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTAT TTTTCTAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATG CTTCAATAATA TTGAAAAAGGAAGAGT	73
modified beta-lactamase promoter (BlaMT) promoter: GACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATcTGTTTgTT CTTcTAgAcACATTCAcAcATGTATCCGCTCATGAGACAATAACCCTGATAAATGC TTCAATgAcATTGAaAAAGGAAGAGT	74
beta-lactamase promoter wild type sequence for the -35 region (-41 to -30, M6): AATACATTCAAA	75
beta-lactamase promoter wild type sequence for the -10 region (-17 to -6, M8): CATGAGACAATA	76
beta-lactamase promoter wild type sequence for the start site (-5 to +7, M9): ACCCTGATAAAT	77
beta-lactamase promoter wild type sequence for the +20 to +31 site (M11): TTGAAAAAGGAA	78

IT IS CLAIMED:

1. An isolated nucleic acid regulatory sequence for a cyclin D1 promoter, said regulatory sequence characterized by the ability to regulate expression of a gene
5 operably linked to a cyclin D1 promoter containing said regulatory sequence.
2. The regulatory promoter sequence of claim 1, wherein said sequence is selected from the group consisting of SEQ ID NO.:5, SEQ ID NO.:6 and SEQ ID NO.:8.
- 10 3. An isolated nucleic acid regulatory sequence for a CD40L promoter, said regulatory sequence characterized by the ability to regulate expression of a gene operably linked to a CD40L promoter containing said regulatory sequence.
- 15 4. The regulatory promoter sequence of claim 3, wherein said sequence is selected from the group consisting of SEQ ID NO.:12, SEQ ID NO.:13, SEQ ID NO.:14 and SEQ ID NO.:15.
- 20 5. An isolated nucleic acid regulatory sequence for an HBV promoter, said regulatory sequence characterized by the ability to regulate expression of a gene operably linked to an HBV promoter containing said regulatory sequence.
6. The regulatory promoter sequence of claim 5 wherein said HBV promoter is a core, preS1 or X promoter.
- 25 7. The regulatory promoter sequence of claim 6 wherein said sequence is the HBV core promoter sequence presented as SEQ ID NO.:20 or SEQ ID NO.:21.
8. The regulatory promoter sequence of claim 6 wherein said sequence is the HBV preS1 promoter sequence presented as SEQ ID NO.:23 or SEQ ID NO.:24.
30
9. The regulatory promoter sequence of claim 6 wherein said sequence is an HBV X promoter sequence selected from the group consisting of SEQ ID NO.:26, SEQ ID NO.:27 and SEQ ID NO.:28.

10. An isolated nucleic acid regulatory sequence for a vancomycin-resistant enterococci (VRE) promoter, said regulatory sequence characterized by the ability to regulate expression of a gene operably linked to a VRE promoter containing said regulatory sequence.

5

11. The regulatory promoter sequence of claim 10 wherein said sequence is selected from the group consisting of SEQ ID NO.:32, SEQ ID NO.:33 and SEQ ID NO.:34.

10

12. An isolated nucleic acid regulatory sequence for an androgen receptor (AR) promoter, said regulatory sequence characterized by the ability to regulate expression of a gene operably linked to an AR promoter containing said regulatory sequence.

15

13. The regulatory promoter sequence of claim 12, wherein said sequence is selected from the group consisting of SEQ ID NO.:64, SEQ ID NO.:65 and SEQ ID NO.:66.

20

14. An isolated nucleic acid regulatory sequence for a HER2 promoter, said regulatory sequence characterized by the ability to regulate expression of a gene operably linked to a HER2 promoter containing said regulatory sequence.

25

15. The regulatory promoter sequence of claim 14, wherein said sequence is selected from the group consisting of SEQ ID NO.:70, SEQ ID NO.:71 and SEQ ID NO.:72.

30

16. An isolated nucleic acid regulatory sequence for a beta lactamase (Bla) promoter, said regulatory sequence characterized by the ability to regulate expression of a gene operably linked to a Bla promoter containing said regulatory sequence.

17. The regulatory promoter sequence of claim 16 wherein said sequence is the Bla promoter sequence presented as SEQ ID NO.77 or SEQ ID NO.78.

35

18. A vector comprising a promoter regulatory nucleic acid sequence of any one of claims 2, 4, 7, 8, 9, 11, 13, 15 and 17.

19. The vector of claim 18, wherein said vector is an expression vector comprising (i) said promoter regulatory nucleic acid sequence operably linked to a promoter and control sequences recognized by a host cell transformed with the vector, and (ii) a transgene encoding an autologous or heterologous gene product.

5

20. The vector of claim 19, wherein said transgene is a reporter gene.

21. A host cell comprising the vector of claim 20.

10

22. The host cell of claim 21, wherein said host cell is a prokaryotic cell.

23. The host cell of claim 21, wherein said host cell is a eukaryotic cell.

24. The host cell of claim 21, wherein said host cell is a mammalian cell.

15

25. A method of regulating gene expression in a cell comprising

(i) introducing into a cell an expression vector according to claim 19,

(ii) exposing said promoter regulatory sequence to a cellular factor or a DNA binding compound resulting in modulated expression of said transgene; and

20

(iii) detecting the expression thereof.

THIS PAGE BLANK (USPTO)

1/14

GCACGTCGCATGGAGACCACCGTGAACGCCACCAATAT

 TGCCCAAGGCTTACATAAGAGGACTCTTGGACTCTCAGC

HNF4

 AATGTCACGACCGACCTTGAGGCATACTTCAAA GACTGT

HNF3-1

HNF

 TTGTTTAAAGACTGGGAGGAGTTGGGGAGGAGATTAGGT

3-2

 TAAAGGCTTTGTACTAGGAGGCTGTAGG CATAAATTGGT

 CTGCGCACCAGCACCATGCAACTTTTTCACCTCTGCCCTAA

Pre-genomic

TCATCTCTTG

* nucleotide conserved at >95% among 75 HBV strains

Fig. 1A

THIS PAGE BLANK (USPTO)

2/14

2701 TTATTATCCAGAACATCTAGGTAAATCATTACTTCCTCCAAACTAGACCACTATTTACACACTCT
HNF1 HNF3

2761 ATGGAAAGGCGGGTATATTATATAAAGAGAGAAACAACACATAGCGCCTCATTTTGTGGGTC
Sp1 TBP RNA Start

2821 ACCATATTCTTGGGAACAAGATCTACAGCATATGGGGC
PreS1 protein start

Fig. 1B

THIS PAGE BLANK (USPTO)

3/14

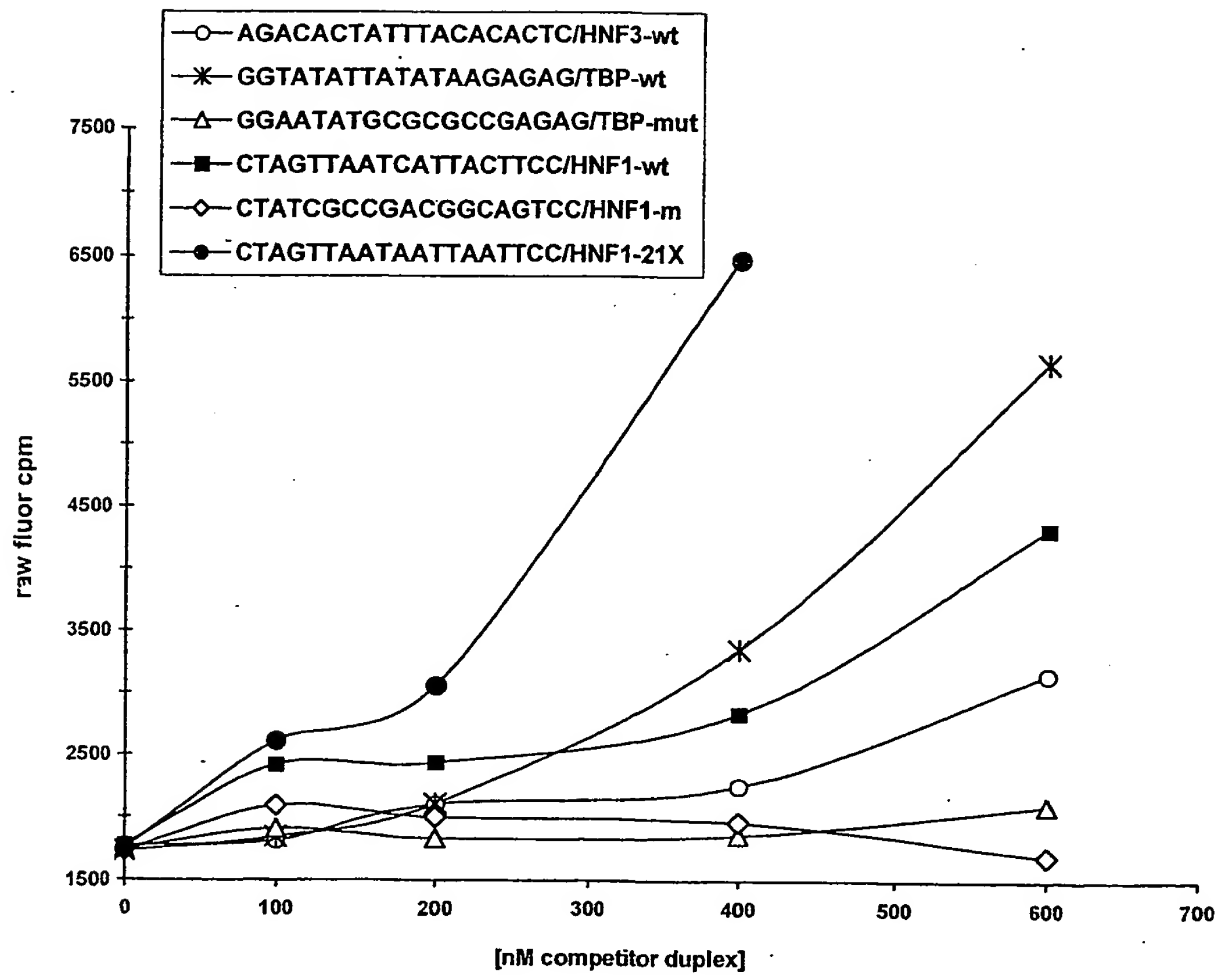


Fig. 2

THIS PAGE BLANK (USPTO)

4/14

1081 CTA AGC AGG CTT TCA CTT TCT CGC CAA CTT ACA AGG CCT TTC TGT GTA AAC AAT
NF1(1100-1119) 2c (1119-1134)

1135 ACC TGA ACC TTT ACC CCG TTG CCC GGC AAC GGC CAG GTC TGT GCC AAG TGT TTG
EF-C(1148-1168)

1189 CTG ACG CAA CCC CCA CTG GCT GGG GCT TGG TCA TGG GCC ATC AGC GCA TGC GTG
E(1180-1202) NF1(1209-1236) X-PBP(1229-1245)

1243 GAA CCT TTT CGG CTC CTC TGC CGA TCC ATA CTG CGG AAC TCC TAG CCG CTT GTT

1297 TTG CTC GCA GCA GGT CTG GAG CAA ACA TTA TCG GGA CTG ATA ACT CTG TTG TCC

1351 TAT CCC GCA AAT ATA CAT CGT TTC CAT GGC TGC TAG 1386

Fig. 3

THIS PAGE BLANK (USPTO)

5/14

CAGCTGGG CCGCCCTTGT GCGCGGGCTG ATGCTCTGAG GCTTGGCTAT
GCGGGGGCCA ACGCGATTGT GGGTGCTCGG GGAGTGGGGG GGGGCACGAC CGTAGGTGCT
CCCTGCTGGG GCAACCCATC GCTCCCCATG CGGAATCCGG GGGTAATTAC CCCCCAGGA
CCCGGAATAT TAGTAATCCT AATTCCCGGC GGGGGAGGGG GCGCGGGAGG AATTCACCCT
GAAAGGTGGG GGTGGGGGGG GTCGCATCTT GCTGTGAGCA CCCTGGCGAA GGGGAGAGGG
CTTTTTCTAT CAGTTTTCTT TGAGCTTTTA CTGTTAAGAG GGTACGGTGG TTTGATGACA
CTGAACTATA TTCAAAGGA AGTAAATGAA CAGTTTTCTT AATTTGGGGC AGGTACTGTA
AAAATAAAAA CAAAAGTTAA GACAGTAAAA TGTCCTTTTA TTTTTTAATG CACCAAAGAG
ACAGAACCTG TAATTTTAAA AACTGTGTAT TTTAATTTAC ATCTGCTTAA GTTTGCGATA
ATATTGGGGA CCCTCTCATG TAACCACGAA CACCTATCGA TTTTGCTAAA AATCAGATCA
GTACACTCGT TTGTTTAATT GATAATTGTT CTGAATTATG CCGGCTCCTG CCAGCCCCCT
CACGCTCACG AATTCAGTCC CAGGGCAAAT TCTAAAGGTG AAGGGACGTC TACACCCCA
ACAAAACCAA TTAGGAACTT CGGTGGTCTT GTCCCAGGCA GAGGGGACTA ATATTTCCAG
CAATTTAATT TCTTTTTTAA TTAATAAAAA TGAGTCAGAA TGGAGATCAC TGTTCCTCAG
CTTTCATTC AGAGGTGTGT TTCTCCCGGT TAAATTGCCG GCACGGGAAG GGAGGGGGTG
CAGTTGGGGA CCCCCGCAAG GACCGACTGG TCAAGGTAGG AAGGCAGCCC GAAGAGTCTC
CAGGCTAGAA GGACAAGATG AAGGAAATGC TGGCCACCAT CTGGGGCTGC TGCTGGAATT
TTCGGGCATT TATTTTATTT TATTTTTTGA GCGAGCGCAT GCTAAGCTGA AATCCCTTTA
ACTTTTAGGG TTACCCCTT GGGCATTGTC AACGACGCC CTGTGCGCCG GAATGAACT
TGCACAGGGG TTGTGTGCCC GTCTCTCCCC GTCTTGCAT GCTAAATTAG TTCTTGCAAT
TTACACGTGT TAATGAAAT GAAAGAAGAT GCAGTCGCTG AGATTCTTTG GCCGTCTGTC
CGCCCGTGGG TGCCCTCGTG GCGTTCTTGG AAATGCGCCC ATTCTGCCGG CTTGGATATG
GGGTGTCGCC GCGCCCCAGT CACCCCTTCT CGTGGTCTCC CCAGGCTGCG TGCTGTGCCG
GCCTTCCTAG TTGTCCCCTA CTGCAGAGCC ACCTCCACCT CACCCCTAA ATCCCGGGGG
ACCACTCGA GCGGACGGG GCCCCCTGCA CCCCTCTTCC CTGGCGGGGA GAAAGGCTGC
AGCGGGGCGA TTTGCATTTT TATGAAAACC GGACTACAGG GGCAACTCCG CCGCAGGGCA
GGCGCGGCGC CTCAGGGATG GCTTTTGGGC TCTGCCCTC GCTGCTCCCG GCGTTTGGCG
CCCGCGCCCC CTCCCCCTGC GCGCGCCCC GCGCCCTCC CGCTCCCAT CTCTGCCGGG
CTTTGATCTT TGCTTAACAA CAGTAACGTC ACACGGACTA CAGGGGAGTT TTGTTGAAGT
TGCAAAGTCC TGGAGCCTCC AGAGGGCTGT CGGCGCAGTA GCAGCGAGCA GCAGAGTCCG
CACGCTCCGG CGAGGGGCAG AAGAGCGCGA GGGAGCGCGG GGCAGCAGAA GCGAGAGCCG
AGCGCGGACC CAGCCAGGAC CCACAGCCCT CCCCAGCTGC CCAGGAAGAG CCCCCA

Fig. 4

THIS PAGE BLANK (USPTO)

6/14

10	20	30	40	50	60	70
GAATTCAC	GGGAGAG	TCAGGAAG	GACAACAG	TAATAGGT	ACAGAGTA	AGAGAGGT
CTTAAGTG	ACCTCTCG	AGTCCTTC	CTGTTGTC	ATTATCC	TGTCTCAT	TCTCTCC
80	90	100	110	120	130	140
CTAAAAAT	ACTCTAAG	GTATTCAG	AAAACATA	TTGAGCTA	AATGGTGG	TCAATTC
GATTTTAT	TGAGATT	CATAAGTC	TTTGTATA	AACTCGAT	TTACCACC	AGTTAAAG
150	160	170	180	190	200	210
GGGAATAT	TGGGCAGA	TCAGACTG	GGAGGCTG	GATCAAGA	TTGAGGCA	GAGGTTGG
CCCTTATA	ACCCGTCT	AGTCTGAC	CCTCCGAC	CTAGTTCT	AACTCCGT	CTCCAAC
220	230	240	250	260	270	280
AACAAC	TTTTCAAG	GGTCACGT	ACAAATCT	GACCTTC	CTCCCCTC	TCGGGTCT
TTGTTGAC	AAAAGTTC	CCAGTGC	TGTTTAGA	CTGGAAG	GAGGGGAG	AGCCAGA
290	300	310	320	330	340	350
GCTGAGCT	TTGCAGGG	CCTGCAGC	TGGCACTC	AAGTTGT	AACTGAC	TGCAGAAG
CGACTCGA	AACGTCCC	GGACGTC	ACCGTGAG	TTCAACA	TTTGA	ACGTCTTC
360	370	380	390	400	410	420
CTTGAGCC	TTTTGGCT	CATGATA	TTCTTCAG	GGAAC	TTACTTGT	AAGAACCA
GAACTCGG	AAAACCGA	GTACTATT	AAGGAAGT	CCTTGATT	AATGAACA	TTCTTGGT
430	440	450	460	470	480	490
GCCTCTGA	TGACTGAT	AAGTTCAT	CGTGCA	AGCCACCT	TTGGCAG	TAGTGAAA
CGGAGACT	ACTGACTA	TTCAAGTA	GCACGTA	TCGGTGG	AACCGTCT	ATCACTTT
500	510	520	530	540	550	560
CTACATAG	CTGGGCC	GACAGGAT	TGGGGCGT	GAGGGGA	AAGCAGGT	TAATATAT
GATGTATC	GACCCGGG	CTGTCCTA	ACCCCGCA	CTCCCCTT	TTCGTCC	ATTGATAT
570	580	590	600	610	620	630
GATAGCAT	CTATCAG	AGTTTTT	TTTCCTAT	GTCTCTCA	ACAATTTT	AGGAATCA
CTATCGTA	GATAGTCT	TCAAAAAT	AAAGGATA	CAGAGAGT	TGTTAAA	TCCTTAGT
640	650	660	670	680	690	700
AAAGCAAT	TATCATGG	TCTAGACC	GTTTGGAT	GAGGTAGG	TTCCACAG	TGCTTTAG
TTTCGTAA	ATAGTACC	AGATCTGG	CAAACCTA	CTCCATCC	AAAGGTGT	ACGAAAAT
710	720	730	740	750	760	770
TTGAAGGA	TCTGATA	TGATGCA	GCCCTTC	AATGTGTA	CCTACACA	TCAGTGAT
AACTTCCT	AGACTATT	ACTACGTT	CGGGAAGT	TTACACAT	GGATGTGT	AGTCACTA
780	790	800	810	820	830	840
AATTCATT	CAAAACTT	GGTGT	ATATTGTT	TGTTCA	GTTTTTAC	ACATGTA
TTAAGTAA	GTTTTGA	CCACAAA	TATAACA	ACAAGTAA	CAAAAATG	TGTACATT
850	860	870	880	890	900	910
AGTTGGCA	TATTTGTT	ACTCATGT	TAGGCTAA	AAATTC	AAATTCAG	TGAGAATT
TCAACCGT	ATAACAAT	TGAGTACA	ATCCGATT	TTTAAGGT	TTTAAGTC	ACTCTTAA

Fig. 5A

THIS PAGE BLANK (USPTO)

7/14

920	930	940	950	960	970	980
TTATTGCTTA	ACGTGTGTCA	AATTTCTTCC	ATGCACATCT	TTATTAGATC	TTCACAGCAA	CCTACAGGAT
AATAACGAAT	TGCACACAGT	TTAAAGAAGG	TACGTGTAGA	AATAATCTAG	AAGTGTCTGT	GGATGTCCTA
990	1000	1010	1020	1030	1040	1050
AAGCAAGACA	GGTGCAAGTG	CCTCCTTTGG	GTATGAGGAA	ACTGAGGTCT	AAAGAGATGA	AGTGATTTGC
TTCGTTCTGT	CCACGTTTAC	GGAGGAAACC	CATACTCCTT	TGACTCCAGA	TTTCTCTACT	TCACTAAACG
1060	1070	1080	1090	1100	1110	1120
CCAAGGCTCA	TAGCAATTTA	TTGGTAGAGC	AAAGACTAGA	ATTCTCTTAA	CTGCAGCCTA	TTTTCCCTAT
GGTTCCGAGT	ATCGTTAAAT	AACCATCTCG	TTTCTGATCT	TAAGAGAATT	GACGTCGGAT	AAAAGGGATA
1130	1140	1150	1160	1170	1180	1190
TCTGAACTGT	TACATCAGCA	TCAACAATTA	TCTAATGGAT	TGGAACAGTG	TACACAGGCA	GCTTAGCTAC
AGACTTGACA	ATGTAGTCGT	AGTTGTTAAT	AGATTACCTA	ACCTTGTCAC	ATGTGTCCGT	CGAATCGATG
1200	1210	1220	1230	1240	1250	1260
GTCAAGTCAC	GATTTTTACT	TTAACTTCAA	TTCCAGAGTC	TTGGCCTGAT	TTCCCTCAAG	ACCCACTTTA
CAGTTCAGTG	CTAAAAATGA	AATTGAAGTT	AAGGTCTCAG	AACCGGACTA	AAGGGAGTTC	TGGGATGAAT
1270	1280	1290	1300	1310	1320	1330
TCTTTGGCTT	TGGAAAATTT	ATTTTTCTTG	CATTATCTTT	CCAGCTAAAT	TTTATTTAAT	AACCATCAGC
AGAAACCGAA	ACCTTTTAAA	TAAAAAGAAC	GTAATAGAAA	GGTCGATTTA	AAATAAATTA	TTGGTAGTCG
1340	1350	1360	1370	1380	1390	1400
ATGCTTTTTT	TGCTTTATGC	CATGTAGACT	TGACCTGAAA	ACCTGCCAGG	CTTTCATTGA	GTTTAGTGAT
TACGAAAAAA	ACGAAATACG	GTACATCTGA	ACTGGACTTT	TGGACGGTCC	GAAAGTAACT	CAAATCACTA
1410	1420	1430	1440	1450	1460	1470
TAAAGAAGTA	AAGTTCTGAG	AAGCAATTAG	TTGATGGGAC	ACCAGTCATA	AAATCAATCC	AAACTTTTGT
ATTTCTTCAT	TTCAAGACTC	TTCGTTAATC	AACTACCCTG	TGGTCAGTAT	TTTAGTTAGG	TTTGAAAACA
1480	1490	1500	1510	1520	1530	1540
TGACATGTGT	TTCTTTCTCC	ATATAACCAGG	TTCCCGCTTC	GTATTAGTAA	GATTGAAAT	GAAATAAGTC
ACTGTACACA	AAGAAAGAGG	TATATGGTCC	AAGGGCGAAG	CATAATCATT	CTAACTTTAA	CTTTATTTCAG
1550	1560	1570	1580	1590	1600	1610
TATTGCTGGT	GGATGAATTT	GTCACTTTCC	TTGAAACTGG	TGAACCCAAA	AAGTTAGACA	GTGATAGGAA
ATAACGACCA	CCTACTTAAA	CAGTGAAAGG	AACTTTGACC	ACTTGGGTTT	TTCAATCTGT	CACTATCCTT
1620	1630	1640	1650	1660	1670	1680
AATACTGCCA	TTGTCTGTTA	AGAAGTCTAT	GACATTTCAA	GGCAAGAATG	AATATATGGA	AGAAGAAACT
TTATGACGGT	AACAGACAAT	TCTTCAGATA	CTGTAAAGTT	CCGTTCTTAC	TTATATACCT	TCTTCTTTGA
1690	1700	1710	1720	1730	1740	1750
TGTTTCTTCT	TTACTTACAA	AAAGGAAAGC	CTGGAAGTGA	ATGATATGGG	TATAATTAAA	AAAAAAAAAA
ACAAAGAAGA	AATGAATGTT	TTTCCTTTTC	GACCTTCACT	TACTATACCC	ATATTAATTT	TTTTTTTTTT
1760	1770	1780	1790	1800	1810	1820
AAAACAAAAA	ACCTTTACGT	AACGTTTTTC	TGGGAGAGAA	GACTACGAAG	CACATTTTCC	AGGAAGTGTG
TTTTGTTTTT	TGGAAATGCA	TTGCAAAACG	ACCCTCTCTT	CTGATGCTTC	GTGTAAAAGG	TCCTTCACAC

Fig. 5B

THIS PAGE BLANK (USPTO)

8/14

1830	1840	1850	1860	1870	1880	1890
GGCTGCAACG	ATTGTGCGCT	CTTAACTAAT	CCTGAGTAAG	GTGGCCACTT	TGACAGTCTT	CTCATGCTGC
CCGACGTTGC	TAACACGCGA	GAATTGATTA	GGACTCATTG	CACCGGTGAA	ACTGTCAGAA	GAGTACGACG
1900	1910	1920	1930	1940	1950	1960
CTCTGCCACC	TTCTCTGCCA	GAAGATACCA	TTTCAACTTT	AACACAGCAT	GATCGAAACA	TACAACCAAA
GAGACGGTGG	AAGAGACGGT	CTTCTATGGT	AAAGTTGAAA	TTGTGTCGTA	CTAGCTTTGT	ATGTTGGTTT
1970	1980	1990	2000	2010	2020	2030
CTTCTCCCCG	ATCTGCGGCC	ACTGGACTGC	CCATCAGCAT	GAAAATTTTT	ATGTATTTAC	TTACTGTTTT
GAAGAGGGGC	TAGACGCCGG	TGACCTGACG	GGTAGTCGTA	CTTTTAAAAA	TACATAAATG	AATGACAAAA
2040	2050	2060	2070	2080	2090	2100
TCTTATCACC	CAGATGATTG	GGTCAGCACT	TTTTGCTGTG	TATCTTCATA	GAAGGCTGGA	CAAGGTAAGA
AGAATAGTGG	GTCTACTAAC	CCAGTCGTGA	AAAACGACAC	ATAGAAGTAT	CTTCCGACCT	GTTCCATTCT
2110	2120	2130	2140	2150	2160	2170
TGAACCACAA	GCCTTTATTA	ACTAAATTTG	GGGTCCTTAC	TAATTCATAG	GTTGGTTCTA	CCCAAATGAT
ACTTGGTGTT	CGGAAATAAT	TGATTTAAAC	CCCAGGAATG	ATTAAGTATC	CAACCAAGAT	GGGTTTACTA
2180	2190	2200	2210	2220	2230	2240
GGATGATGGT	AGAAACCAAA	TAGAAGAATG	GTCTTGTGGC	ATAATGTTTG	TTCCCTAGTC	AATGAAGTCT
CCTACTACCA	TCTTTGGTTT	ATCTTCTTAC	CAGAACACCG	TATTACAAAC	AAGGGATCAG	TTACTTGAGA
2250	2260	2270	2280	2290	2300	2310
CATATTCTTG	TCTCTGGTTA	GGATCTTGGG	ATCTGGAGTC	AGACTGCCTG	GGCTCAAATC	TTGGCTCTGC
GTATAAGAAC	AGAGACCAAT	CCTAGAACCC	TAGACCTCAG	TCTGACGGAC	CCGAGTTTAG	AACCGAGACG
2320	2330	2340	2350	2360	2370	2380
CCATACCATC	TCTGTTATCC	TGGGGCAAGT	GCCTCAGTTT	CCACATCTGA	GAAATGGGGA	TGGTAGTGGT
GGTATGGTAG	AGACAATAGG	ACCCCGTTCA	CGGAGTCAAA	GGTGTAGACT	CTTTACCCCT	ACCATCACCA
2390						
GTCCATTTCA	TAGAT					
CAGGTAAAGT	ATCTA					

Fig. 5C

THIS PAGE BLANK (USPTO)

9/14

GAGATGTATATAATTTTTTAGGAAAATCTCAAGGTTATCTTTACTTTTTCTTA
GGAAATTAACAATTTAATATTAAGAAACGGCTCGTTCTTACACGGTAGACTTA
ATACCGTAAGAACGAGCCGTTTTTCGTTCTTCAGAGAAAGATTTGACAAGATTA
CCATTGGCATCCCCGTTTTATTTGGTGCCTTTCACAGAAAGGGTTGGTCTTAA
TT

Fig. 6

THIS PAGE BLANK (USPTO)

10/14

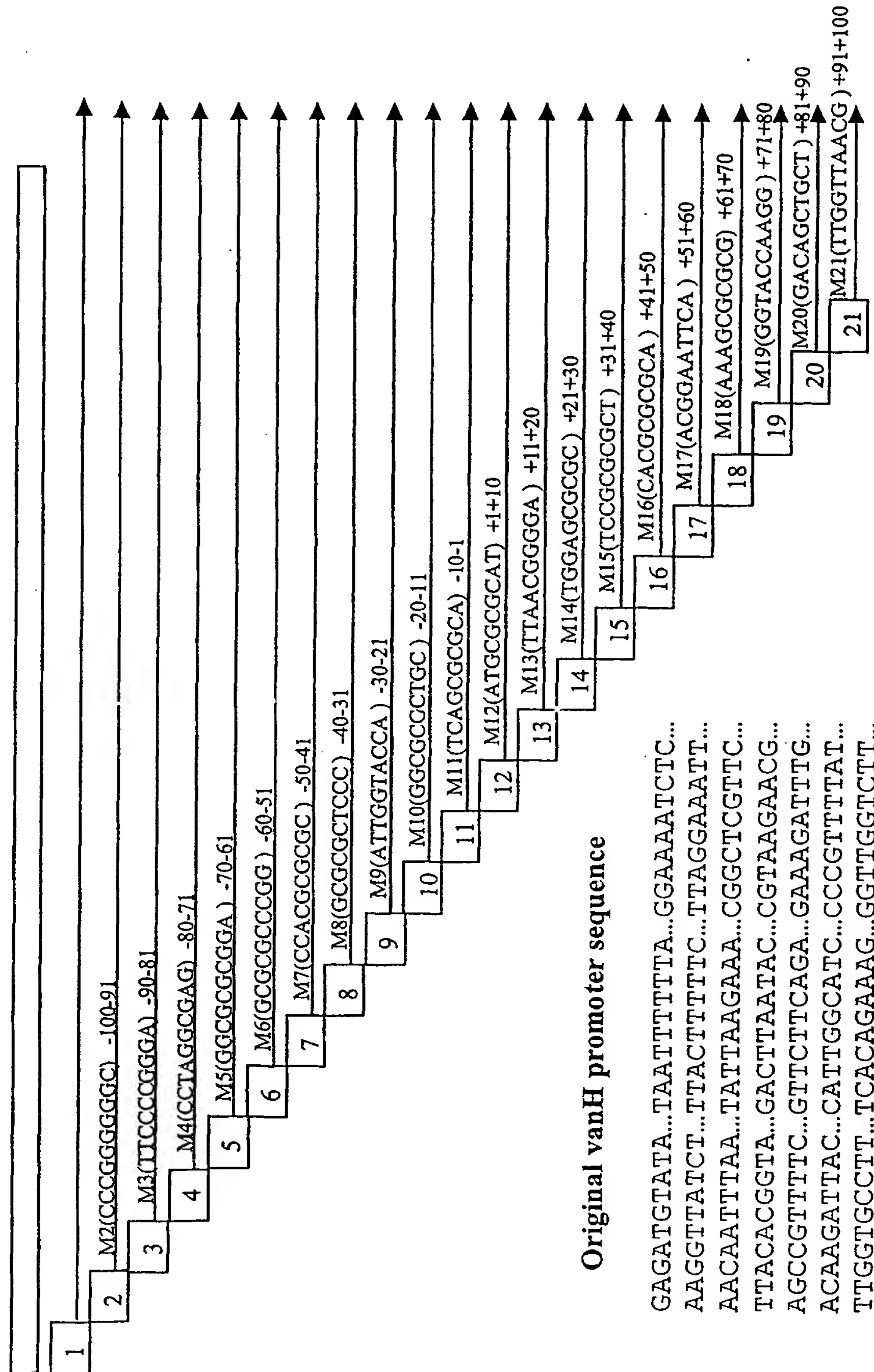


Fig. 7

THIS PAGE BLANK (USPTO)

11/14

TCTAGAAAAT AATTCCCAAT ATTGAATCCC AAAGAATTCA ACATTTGGGC TGTCGTTTGA 61
AAGATAAGTT GAATTTGGTC ATGAAGGAAG AGAGGGGGGA TACAATTTCA GTAAAAGGTA 121
ACAGCAAGGT CCAAAGACAG TCAGGTCTTC AGTAGTATGG AGTATATTCA GAGGGAGCCA 181
AGATGTCTGA TGTGAACATA AAAGATTGGT GGTGGTAGG AGGAAGAGGT GTGAGAAGAG 241
GCTGTAAAGA AAAATTGAAA CTTGATTGTG ATGGACTTTA AAGGCTAGGC TATGGGACTT 301
GGACATGAAT CTGCAGGCCA GTGTTTGCAG ACTGGCGCCC ATAAGTGTCT ATCACAGCAA 361
CACAGACATG TGTGTTTGG CCTGCAGAGG TTTGGCCTGC ATGATGATTT TAAACCATCT 421
GAATTAGTAG CCATCATTTT CAAAAATCAA GAGATGCCAC ATTAAAATAT GGAATGCTGC 481
TGTTCTTGAA AATAATGAAA CATCTGGAAC ATTGAGGCCA CATTCTGAC TGACAGCAAT 541
CAGTTGGAGC TGCGTAGTGA CTGCCCCTT TACATGGGGC ATCTGATCCC TAGTCGATTA 601
CAGCTGCCAC CACTTCCCTT TATCTCTCTA ATACCAAGCT CTTTTCCTC ATTTTGTGTA 661
CTTAAGAGAT ATTTGGGTTT GAAACCTCTG ATGCAGGTAA TTGAGGGTTA TAGAGCAGAG 721
GACAGATGCT ATCAGAGTTG TCTTTTAAGA AAGAACCCTC TGTTCTTCAT TTTGTTGAAG 781
ATAGCCTGGA AGAGGGCAGC CAGGGGAGAA GTTAGGGCTG GAGCTATGAG AAAGCATAAG 841
ATGAGATGAT GGCTTCAACA TTGAGGACAG AAAGAATATT GAGATGAGAA AGTAGTCCAT 901
ATAAGCATCT ATGCAAAGGA AATAGCAGAT GTCCTCAAAT CAGCAGAGGC AACAACCTCTG 961
AAAGTTTATT CATAAGCCCC TCTTTTCATC TCCAATCCAG TTCAAATGTA ATTATTTAAA 1021
TTGTTCTTCA CTCTCCTTCC TGGATCATGA ATGAGCTCCT TAAATGCAGG GTCCACAGTG 1081
TCCTATTCAT CAGTGAATTC CAAGTGCCTA GCACAGAGCC TGGCAAATAG TAAATGCTTA 1141
ACAAATATTC GTTCAGTGCA TGAATTGGAG TGATTCTCTA CTTTGCCTCA TAAGTTGAAA 1201
AAAGGTTTAT TACATACCTA AATATGCTGA AATCACAGGG CATTGGCAA CCCCCAAAA 1261
CCAAACTCC CAGTTTGGAA ACAGAAATTT AATTCTGTGA AAATAAAATC CATTCATTTA 1321
TTCAAAAAAT ATTTATTAAA CAATGACCAT GTCCACACCA GGCTGAGTCC TAAGGATTCA 1381
ATGATGAACA AAAACCAACA TGATTCTGCT TCTTAGGAAA CATAAGTTC AGTGAGGAAA 1441
ACAGATTGTG AGAAGTCCTC CAACAAATAC TGGGTGCTAT TAAAATATAT TAAAAGGTGA 1501
GTGGGTGAGG GACTTGAGCT AGCCTAGGTG GTTCAGGAAG TCTTCTGGA TGTGCTGATA 1561
TGCATAGGCA TTAAGTAGAT AAATAGAGAG AAGGATGAAC CAACATTGCA GGTAGAGGGA 1621
ACAGAATATG CAAAGGCAGG AAGGATTATG GAGTCGTTGG AGGACCTGAA TAAAGGCCCA 1681
GTGTAAGTGG ATCTCAGAAA ACAGGAGGAA AGGTGTATGA GATGAGATCA GAGAGGCAGA 1741
TCATGTGGGG TATGGTTAAT GTTTTGGACT TTTCTATTAA GAGCAATGGG GAGACAGTGA 1801
CAGGACTTAA ACGGGGAAAT AATATGACCA GATTAACTT TCTAAAAAAC CCTCTATGCA 1861
AATATATATT GAGAGTTAAT TATTGACAAA GATTCAAAG CAACAAAGTG GAGAGAGAAT 1921
AGTATTTTCA AAAAATGGTG CCAAACAAT AGGACATCTA TATTAAGAGT TGGGTATCTG 1981
TCTACAAAAC TTAATTCAAA ATGGATCACA GACCTAAATG TAAAGTGAAG AGCTATACAA 2041
CTTCTGGAAG GAAAACACAG ATGGGAATCT GTGTGATCTT GAGTTTGAAA ATGATTTTAT 2101
ATATCTGACA CCATAATCCG TAAGTTAACA TAATTCATAA GTGAACAAAG TGATGAAGTGA 2161
GACTTCATCA GAATTTAAAA TGTTTGTGCT TCAAAAGACA CTGGTATGAT AATGAAGACA 2221
AACTACAGAT AAGATATTGT TGAATCATAT TTCTGATAAA GGAATTGTGG CTCAGAATAC 2281
ATAACTCTAA ACCCCCATAA TAAATTACAA GTAGCCCAAT TAAAAAAGTA AAAAGAGAAA 2341
AAATTTACAG TCTTCATCAA AGAAAGTATC AATTGTAAAA TAAGCACATG AAAAATGCTC 2401
TGCATCTTTA TTCATGGGGG GATGAAATAA AAATTAAATG GGAAAGACAC CTCTAATTAG 2461
AATACTAAAA TTAAGGAGAC TGACCATAAC AAGTATTGGT GAAGTGGAAA TGTAAAATGA 2521
TACAATCAAC TTAGGTAGAT GATTTGGAAG TTTCTTACAA AAGTAGGTGT ATACCTACCC 2581
TGTGACTCAC CCATTCCATG GCTAAGTATT TACCTGAGAG AAATGAAAGA ATACATCCAT 2641
ACAAAGATGT TTATACAAAT ATTTATAGCA GTTTTATTTG TAGTAGCCCC AACTGAAAA 2701
GAACCCAAAT GTCCATCAAA AGTGAATGGA TAAACAAAGC GTGGTACAGC AATGCAATAG 2761
AATACTACTT AGCAATAAAG AAGAATGAGC TAGTGATATA CATAACAGCT TAAATGTACA 2821
TCAAAGGCAT TGTGCTCAGT GAAAGATGCA AGTAAAAAAA AAAAAGAGTA CATGCTGTAT 2881
AGTTCCATTG ACATAAACT CTGGAAAGTG AAAACAGTC TATACTGACA GAAAGCAGAT 2941
CATTGGTTGC CTGAGGAGGA GGAGTATAGG AGAGGTGGAG GGAAAATGTA CAAAGTGGCA 3001
CAATAAAAAC TTTTGAATC ATAGATATAT TCACTATCTT GATTGAGTGA TGATTTTCATG 3061

Fig. 8A

THIS PAGE BLANK (USPTO)

12/14

```

AGTGCACGTG CGTGTGTCAA AAATGATCAA TTTATGCAAC TTAAATATG TGCAGTTTAT 3121
TGTATATATC AATTATACCT CAGTACGGCT ATTAAAAAGA AACCTCTGG CTGCACAATG 3181
CAGAACTGAT TCTAGGAAAG AGTGGAGGGA GGATGACCAT TTACAGTGCT CCAGGTGGAA 3241
GAGAACGGTG CCTTCTGGAA GTGAACTAGG TTGGCAACAA CAGAGATGAA ATAAATGGGC 3301
AGATGTGTGA GATACTTAGG AAATAAAACC CGATGGTCAC CATTTTCCAA AGGTCAGCTC 3361
ATCCTGGCTT TCCAGAGCAA AGAGCTAGGG AAGACTTTAT TAATAAATCC CTCTTGAAGT 3421
TGCAGAGGAA GCTTATAGCA GAAACTTACT CTCAACCTGA CTAATCTGAG AGAACACCTC 3481
TGGTTCCATT TGATTACTAA AAAACTGCAA AGAACAGGAG GAGAAAGAAG AAGAAAGCTG 3541
GTACAAACAG TGAACCTATA TAATATTAAT CAATAATTGT CTCTTGTTCT TAAAAGCAAT 3601
GGGAAGAAAA TGAGATTTGA GCTGGAAGAT CAGAGTTCAA AATCCAAATA AAGTATATGG 3661
CCCTAATATG CTTATAGTAG TTAACCTTTC CTGATAATGA TATAATTGTT GACAGCACCA 3721
TCTTTAAAT AAAATAACAT AGTAATCCTT CAGATTTGTA GAAGATCTTT CCTGTTTACA 3781
AGTTTGTCT ATACACATTA TGTCTTTTAA ATGACACACT AGCCTTCTGA GGGTAACTTA 3841
TATTGGCAAC AGTTTTCAGA TGTGGAAACT GTGAAGACAA TGTGTTGAT GTGGAAGCAA 3901
CATAAACTTT GGAGTCTTTC AGACCCAGGT TTGAATGTCA GACTGCTTTT TATTCAGAGT 3961
AACTTCAGAG CATTATTTCT CACCTTAATT TTTTTTCAGG CCTCTTGTG TCTATGTGTC 4021
CTCTTCACTC CTGTCCATTG TTTCTTCAGT GATTTTTGCC ACCTTCCTTC ACTGTTAGTG 4081
TGTAGACACA TAGTTCTCCT GGCTCTGAGA GCCTATGTTA ATTCCATTCT ACCATCCTGC 4141
CACGGCCAC TCAATTCCTA TTGAGCAATG CTAGTTGAAA GTTGTGGTGG GATTAAATGT 4201
TGCAATGAGT ATTCAAATGA GGTTGAAGTA TCTACGCATT CTACTTACAT ATGGTGAGGT 4261
ATATTCAAGG AAGCTGTAGC CATTAAAATC TCAGGAAATA ATTTTTCACC TCCTCAGGTG 4321
AAAGGGTCTT CAGGCCTTG TGTCTGGAA GGTTCAATTA TAGCCATTTC CCAAATGACA 4381
ATGCGATTGA TGAGTCTAGA GTCTAGCTCA AATAGCAATG GACTGGAAGA CTAGTTTAGG 4441
TTTTACTAAT GTGGAACATA GAACAAATTA TGTCCTTGTT TCAGCCTGTT CATCTGTGAA 4501
ATAGAGCCTA TCATATCCAG TCTTCCTTGC CTTTAGGTTT GAGTTACCTT CTTTGGTCAA 4561
GGTAAGTAAA TGCCTATGAT GTTTGGCTGT GCACAAGATA AAGCTACAAC AAAGCTACAA 4621
CCCATCTTTT CTCTGTAGAA GACTCAAAAA GCAAAAGAGA CCCAGGAAAA TCTCGGAATG 4681
ACTTTTGGAA CAGAGAGCCT CCCAGAAATC AGAAGTCAAG GAATTTAAAC ATAGGGAAGG 4741
CCCAGGTCTC TACTGACATA AAGGAAAGAT GTTTTCTTAT AGGTTTCACG TTTACATTTT 4801
CTCTCTCTTG ATCCCATTCC CACTTGCATC TGCCACCTTT ACACAGGGCT TATGGGACCT 4861
CCTCCACAAA AGAGCAGTTG CAGTAACCCA CATCATCCTC TACGCCCTGG CTGTCCATCA 4921
AGAGGCGAAA AGCAGCCCTA TATAGTTTCT ATCCTTGGAT AGTTCCAGTT GTAAAGTTTA 4981
AAATATGCGA AGGCAACTTG GAAAAGCAAG CGGCTGCATA CAAAGCAAAC GTTTACAGAG 5041
CTCTGGACAA AATTGAGCGC CTATGTGTAC ATGGCAAGTG TTTTGTAGTGT TTGTGTGTTT 5101
ACCTGCTTGT CTGGGTGATT TTGCCTTTGA GAGTCTGGAG AGTAGAAGTA CTGGTTAAAG 5161
GAACTTCCAG ACAGGAAGAA GGCAGAGAAG AGGGTAGAAA TGAATCTGAT TCTTGGGGCT 5221
GAGGGTTTCT AGAGCAAATG GCACAATGCC ACGAGGCCCG ATCTATCCCT ATGACGGAAT 5281
CTAAGGTTTC AGCAAGTATC TGCTGGCTTG GTCATGGCTT GCTCCTCAGT TTGTAGGAGA 5341
CTCTCCCACT CTCCCATCTG CGCGCTCTTA TCAGTCCTGA AAAGAACCCC TGGCAGCCAG 5401
GAGCAGGTAT TCCTATCGTC CTTTTCTTCC CTCCCTCGCC CCACCCTGTT GGTTTTTTAG 5461
ATTGGGCTTT GGAACCAAAT TTCCTGAGTG CTGGCCTCCA GGAAATCTGG AGCCCTGGCG 5521
CCTAAACCTT GGTTTAGGAA ACCAGGAGCT ATTCAGGAAG CAGGGGTCTT CCAGGGCTAG 5581
AGCTAGCCTC TCCTGCCCTC GCCACGCTG CGCCAGCACT TGTTTCTCCA AAGCCACTAG 5641
GCAGGCGTTA GCGCGCGGTG AGGGGAGGGG AGAAAAGGAA AGGGGAGGGG AGGGAAAAGG 5701
AGGTGGGAAG GCAAGGAGGC CGGCCCGGTG GGGGCGGGAC CCGACTCGCA AACTGTTGCA 5761
TTTGCTCTCC ACCTCCCAGC GCCCCCTCCG AGATCCCGGG GAGCCAGCTT GCTGGGAGAG 5821
CGGGACGGTC CGGAGCAAGC CCACAGGCAG AGGAGGCGAC AGAGGGAAAA AGGGCCGAGC 5881
TAGCCGCTCC AGTGCTGTAC AGGAGCCGAA GGGACGCACC ACGCCAGCCC CAGCCCGGCT 5941
CCAGCGACAG CCAACGCCTC TTGCAGCGCG GCGGCTTCGA AGCCGCCGCC CGGAGCTGCC 6001
CTTTCCTCTT CGGTGAAGTT TTTAAAAGCT GCTAAAGACT CGGAGGAAGC AAGGAAAGTG 6061

```

Fig. 8B

THIS PAGE BLANK (USPTO)

13/14

```
CCTGGTAGGA CTGACGGCTG CCTTTGTCCT CCTCCTCTCC ACCCGCCTC CCCCCACCCT 6121
GCCTTCCCCC CCTCCCCCGT CTTCTCTCCC GCAGCTGCCT CAGTCGGCTA CTCTCAGCCA 6181
ACCCCCCTCA CCACCCTTCT CCCCACCCGC CCCCCCGCCC CCGTCGCCCA GCGCTGCCAG 6241
CCCGAGTTTG CAGAGAGGTA ACTCCCTTTG GCTGCGAGCG GGCGAGCTAG CTGCACATTG 6301
CAAAGAAGGC TCTTAGGAGC CAGGCGACTG GGGAGCGGCT TCAGCACTGC AGCCACGACC 6361
CGCCTGGTTA GGCTGCACGC GGAGAGAACC CTCTGTTTTT CCCCCTCTC TCTCCACCTC 6421
CTCCTGCCTT CCCCACCCCG AGTGCGGAGC CAGAGATCAA AAGATGAAAA GGCAGTCAGG 6481
TCTTCAGTAG CCAAAAAACA AAACAAACAA AAACAAAAAA CAAGAAATAA AAGAAAAAGA 6541
TAATAACTCA GTTCTTATTT GCACCTACTT CAGTGGACAC TGAATTTGGA AGGTGGAGGA 6601
TTTTGTTTTT TTCTTTTAAG ATCTGGGCAT CTTTTGAATC TACCCTTCAA GTATTAAGAG 6661
ACAGACTGTG AGCCTAGCAG GGCAGATCTT GTCCACCGTG TGTCTTCTC TGCACGAGAC 6721
TTTGAGGCTG TCAGAGCGCT TTTTGCGTGG TTGCTCCCGC AAGTTTCCTT CTCTGGAGCT 6781
TCCCGCAGGT GGGCAGCTAG CTGCAGCGAC TACCGCATCA TCACAGCCTG TTGAACTCTT 6841
CTGAGCAAGA GAAGGGGAGG CGGGGTAAGG GAAGTAGGTG GAAGATTCAG CCAAGCTCAA 6901
GGATG
```

Fig. 8C

THIS PAGE BLANK (USPTO)

14/14

CA GGCCCCACAA AACCTAGATC TGCCCCAGTA TAACTAAATC 1501
TGGGACCATT TATTGAGCAA TTATTATGTG CCAAGTATTG CGCTGAGTGC TTCCAGAGCA 1561
TTATCTCCTT TAACCCAGC ATAGTATGTC AGATGCTGTT TTACAGATGA GCCAACTGAG 1621
ACCAGAGATG CTCAGTCACT TGCCCAAGGT GACATGACTG ATATGGAATA GAGTCAAGAT 1681
TTTTTTTTTT TTTTTTGACA CGGAGTCTCA CTCTGTCTCC CAGGCTGGAG TGCAGAGGCG 1741
CAATCTCAGC TCACTGCAAG CTCTGCCTCC CAGGTTACAG CATTCTCCTG CCTCAGCCTC 1801
CTGAGTAGCT GGGACTACAG GCACCCGCCA CCACACCTGG CTAATTTTTT GTATTTTTAG 1861
CAGAGACAGG GTTTCACCGT GTTAGCCAGG ATGGTCTCGA TCTCCTGACC TCGTGATCTG 1921
CCTGCCTCGG CCTCCCAAAG TGATGGAATT ACAGGTGTGA GCCACCGCGA CTGGCCAGAT 1981
TCAAGATTTG AACCCAGGTC CTCTTGGTCC CAGAGGCCCC TGTTTCTCAA CTCCCTAGCA 2041
TGCATACGCA CCTGTCCCTC TAGAGGTGCC TGCTTAAGTG TGCTCAGCAC ATGGAAGCAA 2101
GTTAGAAATG CTAGGTATAC CTGTAAAGAG GTGTGGGAGA TGGGGGGGAG GGAAGAGAGA 2161
AAGAGATGCT GGTGTCCTTC ATTCTCCAGT CCCTGATAGG TGCCTTTGAT CCCTTCTTGA 2221
CCAGTATAGC TGCATTCTTG GCTGGGGCAT TCCAAC TAGA ACTGCCAAAT TTAGCACATA 2281
AAAATAAGGA GGCCAGTTA AATTTGAATT TCAGATAAAC AATGAATAAT TTGT TAGTAT 2341
AAATATGTCC CATGCAATAT CTTGTTGAAA TTAAAAAAA AAAAAAAGT CTTCTTCCA 2401
TCCCCACCCC TACCACTAGG CCTAAGGAAT AGGGTCAGGG GCTCCAAATA GAATGTGGTT 2461
GAGAAGTGGA ATTAAGCAGG CTAATAGAAG GCAAGGGGCA AAGAAGAAAC CTTGAATGCA 2521
TTGGGTGCTG GGTGCCTCCT TAAATAAGCA AGAAGGGTGC ATTTTGAAGA ATTGAGATAG 2581
AAGTCTTTTT GGGCTGGGTG CAGTTGCTCG TGGTTGTAAT TCCAGCACTT TGGGAGGCTG 2641
AGGCGGGAGG ATCACCTGAG CTTGGGAGTT CAAGACCAGC CTCACCAACG TGGAGAAACC 2701
CTGTCTTTAC TAAAAATACA AAAAATTCAG CTGGTCATGG TGGCACATGC CTGTAATCCC 2761
AGCTGCTCGG GAGGCTGAGG CAGGAGAATC ACTTGAACCA GGGAGGCAGA GGTGTGTGGT 2821
AGCAGAGATC GCGCCATTGC TCTCCAGCCT GGGCAACAAG AGCAAAAGTT CGTTTAAAAA 2881
AAAAAAAAG TCCTTTTCGAT GTGACTGTCT CCTCCAAAT TTGTAGACCC TCTTAAGATC 2941
ATGCTTTTCA GATACTTCAA AGATTCCAGA AGATATGCC CGGGGGTCCT GGAAGCCACA 3001
AGGTAAACAC AACACATCCC CCTCCTTGAC TATCAATTTT ACTAGAGGAT GTGGTGGGAA 3061
AACCATTATT TGATATTAAA ACAATAGGCT TGGGATGGAG TAGGATGCAA GCTCCCCAGG 3121
AAGTTAGATA ACTGAGACTT AAAGGGTGTT AAGAGTGGCA GCCTAGGGAA ATTTATCCCC 3181
GACTCCGGGG GAGGGGGCAG AGTCACCAGC CTCTGCATTT AGGGATTCTC CGAGGAAAAG 3241
TGTGAGAACG GCTGCAGGCA ACCCAGGCGT CCCGGCGCTA GGAGGGACGA CCCAGGCCTG 3301
CGCGAAGAGA GGGAGAAAGT GAAGCTGGGA GTTGCCGACT CCCAGACTTC GTTGGAATGC 3361
AGTTGGAGGG GGCAGCTGG GAGCGCGCTT GCTCCCAATC ACCGGAGAAG GAGGAGGTGG 3421
AGGAGGAGGG CTGCTTGAGG AAGTATAAGA ATGAAGTTGT GAAGCTGAGA TTCCCCTCCA 3481
TTGGGACCGG AGAAACCAGG GGAGCCCCC GGGCAGCCGC GCGCCCTTC CCACGGGGCC 3541
CTTTACTGCG CCGCGCGCCC GGCCCCACC CCTCGCAGCA CCGCGCGCCC CGCGCCCTCC 3601
CAGCCGGGTC CAGCCGGAGC CATGG

Fig. 9

THIS PAGE BLANK (USPTO)

SEQUENCE LISTING

<110> Genelabs Technologies, Inc.

<120> PROMOTERS FOR REGULATED GENE EXPRESSION

<130> 4600-0135.41

<140> Not Yet Assigned

<141> Filed Herewith

<150> US 60/209,549

<151> 2000-06-06

<160> 78

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 1903

<212> DNA

<213> Homo sapiens

<400> 1

cagctggggc	gcccttgtgc	gcgggctgat	gctctgaggc	ttggctatgc	gggggccaac	60
gcgattgtgg	gtgctcgggg	agtggggggg	ggcagcaccg	taggtgctcc	ctgctggggc	120
aacccatcgc	tcccatgcg	gaatcggggg	gtaattaccc	ccccaggacc	cggaatatta	180
gtaatcctaa	ttcccggcgg	gggagggggc	gcgggaggaa	ttcacccctga	aaggtggggg	240
tggggggggg	cgcctcttgc	tgtgagcacc	ctggcgaagg	ggagagggct	ttttctatca	300
gttttctttg	agcttttact	gttaagaggg	tacggtggtt	tgatgacact	gaactatatt	360
caaaaggaag	taaatgaaca	gttttcttaa	tttggggcag	gtactgtaaa	aataaaaaca	420
aaagttaaga	cagtaaaatg	tccttttatt	ttttaatgca	ccaaagagac	agaacctgta	480
attttaaaaa	ctgtgtatgt	taatttacat	ctgcttaagt	ttgcgataat	attggggacc	540
ctctcatgta	accacgaaca	cctatcgatt	ttgctaaaaa	tcagatcagt	acactcgttt	600
gtttaattga	taattgttct	gaattatgcc	ggctcctgcc	agccccctca	cgctcacgaa	660
ttcagtccca	gggcaaattc	taaaggtgaa	gggacgtcta	cacccccaac	aaaaccaatt	720
aggaacttcg	gtggtcttgt	cccaggcaga	ggggactaat	atthccagca	atttaatttc	780
ttttttaatt	aaaaaaaatg	agtcagaatg	gagatcactg	tttctcagct	ttccattcag	840
aggtgtgttt	ctcccgggta	aattgccggc	acgggaaggg	agggggtgca	gttggggacc	900
cccgaagga	ccgactggtc	aaggtaggaa	ggcagcccg	agagtctcca	ggctagaagg	960
acaagatgaa	ggaaatgctg	gccaccatct	tgggctgctg	ctggaatttt	cgggcattta	1020
ttttatttta	ttttttgagc	gagcgcacgc	taagctgaaa	tccttttaac	ttttaggggt	1080
accccccttg	gcatttgcaa	cgacgccctt	gtgcgcggga	atgaaacttg	cacagggggt	1140
gtgtgcccg	tcctccccgt	ccttgcatgc	taaattagtt	cttgcaattt	acacgtgtta	1200
atgaaaatga	aagaagatgc	agtcgctgag	attctttggc	cgtctgtccg	cccgtgggtg	1260
ccctcgtggc	gttcttgga	atgcgcccc	tctgcgggct	tggatatggg	gtgtcgccgc	1320
gccccagtca	ccccttctcg	tgtctcccc	aggctgcgtg	ctgtgccggc	cttctagttt	1380
gtccctact	gcagagccac	ctccacctca	ccccctaaat	cccgggggac	ccactcgagg	1440
cggacggggc	cccctgcacc	cctcttccct	ggcggggaga	aaggctgcag	cggggagatt	1500
tgcatttcta	tgaaaaccgg	actacagggg	caactccgcc	gcagggcagg	cgcgccgcct	1560
cagggatggc	ttttgggctc	tgccctcgc	tgctcccgcc	gtttggcgcc	cgcgccccct	1620
ccccctgcgc	ccgccccgc	ccccctcccg	ctccattctt	ctgcggggct	ttgatctttg	1680
cttaacaaca	gtaacgtcac	acggactaca	ggggagtttt	gttgaagttg	caaagtcctg	1740
gagcctccag	agggctgtcg	gcgcagtagc	agcgagcagc	agagtccgca	cgctccggcg	1800
aggggcagaa	gagcgcgagg	gagcgcgggg	cagcagaagc	gagagccgag	cgcggaacca	1860
gccaggaccc	acagccctcc	ccagctgccc	aggaagagcc	cca		1903

<210> 2

<211> 32

<212> DNA

THIS PAGE BLANK (USPTO)

<213> Artificial Sequence

<220>

<223> primer

<400> 2

gcacgcgtgc tagccagctg ggccgccctt gt

32

<210> 3

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 3

atccatggaa gctttggggc tcttcctggg ca

32

<210> 4

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 4

gcacgcgtgc tagctggagc ctccagaggg ctgt

34

<210> 5

<211> 10

<212> DNA

<213> Homo sapiens

<400> 5

gagttttgtt

10

<210> 6

<211> 7

<212> DNA

<213> Homo sapiens

<400> 6

gagtttt

7

<210> 7

<211> 16

<212> DNA

<213> Homo sapiens

<400> 7

cagtaacgtc acacgg

16

<210> 8

<211> 10

<212> DNA

<213> Homo sapiens

<400> 8

cctccagagg

10

THIS PAGE BLANK (USPTO)

<210> 9
<211> 2395
<212> DNA
<213> Homo sapiens

<400> 9
gaattcactg gggagagcat tcaggaagat gacaacagga taatagggtca acagagtaat 60
agagaggtcg ctaaaaataa actctaagaa gtattcagcc aaaactatta ttgagctaata 120
aatggtggga tcaatttcag gggaatattg tgggcagaag tcagactgta ggaggctggg 180
gatcaagaag ttgaggcaag gaggttggac aacaactgtt ttttcaagtt ggtcacgtga 240
acaaatctgt gaccttcagc ctccccctccc tcgggtcttg gctgagctga ttgcagggcc 300
cctgcagctc tggcactctc aagttgtata aaactgacag tgcagaagtc cttgagccca 360
ttttggctct catgataatt ttccttcagt ggaactaagg ttacttgtct aagaacccaa 420
gcctctgact tgactgatca aagttcatca cgtgcatcga agccacctac ttggcagatg 480
tagtgaaaag ctacatagat ctgggccag gacaggatgc tggggcggtg gaggggaaga 540
aagcaggtgc taactatata gatagcatgc ctatcagagc agtttttacg tttcctatct 600
gtctctcaa acaattttat aggaatcatc aaagcaattt tatcatggtt tctagaccag 660
gtttggatgt gaggtaggga tttccacagc tgcttttagt ttgaaggaaa tctgataaga 720
tgatgcaaaa gcccttcaga aatgtgtaat cctacacact tcagtgatc aattcattgt 780
caaaacttaa ggtgttttta atattgttat tgttcatttg gtttttacca acatgtaagg 840
agttggcaat tatttgtaa actcatgtct taggctaaat aaattccaaa aaattcagga 900
tgagaattgt ttattgctta acgtgtgtca aatttcttcc atgcacatct ttattagatc 960
ttcacagcaa cctacaggat aagcaagaca ggtgcaagtg cctcctttgg gtatgaggaa 1020
actgaggtct aaagagatga agtgatttgc ccaaggctca tagcaattta ttggtagagc 1080
aaagactaga attctcttaa ctgcagccta tttccctat tctgaactgt tacatcagca 1140
tcaacaatta tctaattgat tggaaacagt tacacaggca gcttagctac gtcaagtcac 1200
gatttttact ttaacttcaa ttccagagtc ttggcctgat ttccctcaag accctactta 1260
tctttggctt tggaaaattt attttcttg cattatctt ccagctaaat tttatttaat 1320
aaccatcagc atgctttttt tgctttatgc catgtagact tgacctgaaa acctgccagg 1380
ctttcattga gtttagtgat taaagaagta aagttctgag aagcaattag ttgatgggac 1440
accagtcata aaatcaatcc aaacttttgt tgacatgtgt ttctttctcc atataccagg 1500
ttcccgttc gtattagtaa gattgaaatt gaaataagtc tattgctggt ggatgaattt 1560
gtcactttcc ttgaaactgg tgaacccaaa aagttagaca gtgataggaa aatactgcca 1620
ttgtctgtta agaagtctat gacatttcaa ggcaagaatg aatataatgga agaagaaact 1680
tgtttcttct ttacttacia aaaggaaagc ctggaagtga atgatatggg tataattaaa 1740
aaaaaaaaa aaaacaaaaa acctttacgt aacgttttgc tgggagagaa gactacgaag 1800
cacattttcc aggaagtgtg ggctgcaacg attgtgcgct cttaactaat cctgagtaag 1860
gtggccactt tgacagtctt ctcatgtgc ctctgccacc ttctctgcca gaagatacca 1920
tttcaacttt aacacagcat gatcgaaaca tacaacaaa cttctccccg atctgcggcc 1980
actggactgc ccatcagcat gaaaattttt atgtatttac ttactgtttt tcttatcacc 2040
cagatgattg ggtcagcact ttttgctgtg tatcttcata gaaggctgga caaggtaaga 2100
tgaaccacaa gcctttatta actaaatttg gggtccttac taattcatag gttggttcta 2160
cccaaagat ggatgatggt agaaaccaa tagaagaatg gtcttgtggc ataattgttg 2220
ttccctagtc aatgaactct catattcttg tctctggtta ggatcttggg atctggagtc 2280
agactgcctg ggctcaaact ttggctctgc ccataccatc tctgttatcc tggggcaagt 2340
gcctcagttt ccacatctga gaaatgggga tggtagtggt gtccatttca tagat 2395

<210> 10
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 10
ttatgatacc tcgaggggag agcattcagg aagatg 36

<210> 11
<211> 36
<212> DNA
<213> Artificial Sequence

THIS PAGE BLANK (USPTO)

<220>

<223> primer

<400> 11

tgaatcacga agcttttgga tcttctggca gagaag

36

<210> 12

<211> 24

<212> DNA

<213> Homo sapiens

<400> 12

gatgaatttg tcactttcct tgaa

24

<210> 13

<211> 20

<212> DNA

<213> Homo sapiens

<400> 13

gacatttcaa ggcaagaatg

20

<210> 14

<211> 35

<212> DNA

<213> Homo sapiens

<400> 14

acatttcaag gcaagaatga atatatggaa gaaga

35

<210> 15

<211> 37

<212> DNA

<213> Homo sapiens

<400> 15

tacgaagcac attttccagg aagtgtgggc tgcaacg

37

<210> 16

<211> 250

<212> DNA

<213> Hepatitis B virus

<400> 16

gcacgtcgca tggagaccac cgtgaacgcc caccaaata tggccaaggt cttacataag

60

aggactcttg gactctcagc aatgtcaacg accgacctg aggcatactt caaagactgt

120

ttgtttaaag actgggagga gttgggggag gagattaggt taaaggctt tgtactagga

180

ggctgtaggc ataaattggt ctgcgcacca gcaccatgca actttttcac ctctgcctaa

240

tcattctctg

250

<210> 17

<211> 12

<212> DNA

<213> Hepatitis B virus

<400> 17

gactgtttgt tt

12

<210> 18

<211> 12

<212> DNA

THIS PAGE BLANK (USPTO)

<213> Hepatitis B virus

<400> 18

aggactcttg ga

12

<210> 19

<211> 15

<212> DNA

<213> Hepatitis B virus

<400> 19

tacttcaaag actgt

15

<210> 20

<211> 23

<212> DNA

<213> Hepatitis B virus

<400> 20

tacttcaaag actgtttgtt taa

23

<210> 21

<211> 15

<212> DNA

<213> Hepatitis B virus

<400> 21

ggctgtaggc ataaa

15

<210> 22

<211> 156

<212> DNA

<213> Hepatitis B virus

<400> 22

ttattatcca gaacatctag ttaatcatta cttccaaact agacactatt tacacactct

60

atggaaggcg ggtatattat ataagagaga aacaacacat agcgcctcat tttgtgggtc

120

accatattct tgggaacaag atctacagca tggggc

156

<210> 23

<211> 15

<212> DNA

<213> Hepatitis B virus

<400> 23

ctagttaatc attac

15

<210> 24

<211> 15

<212> DNA

<213> Hepatitis B virus

<400> 24

ttatataaga gagaa

15

<210> 25

<211> 306

<212> DNA

<213> Hepatitis B virus

<400> 25

ctaagcaggc tttcactttc tcgccaaactt acaaggcctt tctgtgtaaa caatacctga

60

THIS PAGE BLANK (USPTO)

acctttaccc cgttgcccgg caacggccag gtctgtgcca agtggttgct gacgcaaccc 120
 ccaactggctg gggcttggtc atgggccatc agcgcatgcg tggaaccttt tcggctcctc 180
 tgccgatcca tactgcggaa ctctagccg cttgttttgc tcgcagcagg tctggagcaa 240
 acattatcgg gactgataac tctgttgctc tatcccgcaa atatacatcg tttccatggc 300
 tgctag 306

<210> 26
 <211> 21
 <212> DNA
 <213> Hepatitis B virus

<400> 26
 tgtaaacaat acctgaacct t 21

<210> 27
 <211> 21
 <212> DNA
 <213> Hepatitis B virus

<400> 27.
 taccocgttg cccggcaacg g 21

<210> 28
 <211> 21
 <212> DNA
 <213> Hepatitis B virus

<400> 28
 gctgacgcaa cccccactgg c 21

<210> 29
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer

<400> 29
 caccgaagct taagcaggct ttcactttct cg 32

<210> 30
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> primer

<400> 30
 cagtaccgga atgccaagct tcgatg 26

<210> 31
 <211> 214
 <212> DNA
 <213> Vancomycin resistant enterococcus

<400> 31
 gagatgtata taatTTTTTA ggaaaatctc aagggttatct ttactTTTTc ttaggaaatt 60
 aacaatttaa tattaagaaa cggctcgttc ttacacggta gacttaatac cgtaagaacg 120
 agccgttttc gttcttcaga gaaagatttg acaagattac cattggcatc cccgttttat 180
 ttggtgcctt tcacagaaag gggttggtctt aatt 214

THIS PAGE BLANK (USPTO)

<210> 32
 <211> 10
 <212> DNA
 <213> Vancomycin resistant enterococcus

<400> 32
 ttaggaaatt 10

<210> 33
 <211> 10
 <212> DNA
 <213> Vancomycin resistant enterococcus

<400> 33
 tattaagaaa 10

<210> 34
 <211> 10
 <212> DNA
 <213> Vancomycin resistant enterococcus

<400> 34
 cgtaagaacg 10

<210> 35
 <211> 6905
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> promoter

<400> 35
 tctagaaaat aattcccaat attgaatccc aaagaattca acatttgggc tgtcgtttga 60
 aagataagtt gaatttggtc atgaaggaag agagggggga tacaatttca gtaaaaggta 120
 acagcaaggt ccaaagacag tcaggtcttc agtagtatgg agtatattca gagggagcca 180
 agatgtctga tgtgaactaa aaagattggg ggttggtagg aggaagaggt gtgagaagag 240
 gctgtaaaga aaaattgaaa cttgattgtg atggacttta aaggctaggc tatgggactt 300
 ggacatgaat ctgcaggcca gtgtttgcag actggcgccc ataactgtct atcacagcaa 360
 cacagacatg tgttgtttgg cctgcagagg tttggcctgc atgatgattt taaaccatct 420
 gaattagtag ccatcatttt caaaaatcaa gagatgccac attaaaatat ggaatgctgc 480
 tgttcttgaa aataatgaaa catctggaac attgaggcca cattcctgac tgacagcaat 540
 cagttggagc tgcgtagtga ctgcccactt tacatggggc atctgatccc tagtcgatta 600
 cagctgccac cacttccctt tatctctcta ataccaagct cttttcactc atttttgtta 660
 cttaagagat atttgggttt gaaacctctg atgcaggtaa ttgaggggta tagagcagag 720
 gacagatgct atcagagttg tcttttaaga aagaaccctc tgttcttcat tttgttgaag 780
 atagcctgga agagggcgagc cagggggagaa gttagggctg gagctatgag aaagcataag 840
 atgagatgat ggcttcaaca ttgaggacag aaagaatatt gagatgagaa agtagtccat 900
 ataagcatct atgcaaagga aatagcagat gtcctcaaat cagcagaggc aacaactctg 960
 aaagtttatt cataagcccc tcttttcact tccaatccag ttcaaagtga attatttaaa 1020
 ttgttcttca ctctccttcc tggatcatga atgagctcct taaatgcagg gtccacagtg 1080
 tcctattcat cagtgaattc caagtgccta gcacagagcc tggcaaatag taaatgctta 1140
 acaaattatc gttcagtgca tgaattggag tgattctcta ctttgcctca taagttgaaa 1200
 aaagggttat tacataccta aatatgctga aatcacaggg catttggaac cccccaaaa 1260
 ccaaaaactcc cagtttggaac acagaatttt aattctgtga aaataaaatc cattcattta 1320
 ttcaaaaaat atttattaaa caatgaccat gtccacacca ggctgagtc taaggattca 1380
 atgatgaaca aaaaccaaca tgattcctgc tcttaggaaa catacagttc agtgaggaaa 1440
 acagattgtg agaagtcctc caacaaatac tgggtgctat taaaatatat taaaagggtga 1500
 gtgggtgagg gacttgagct agcctagggtg gttcaggaag tcttcctgga tgtgctgata 1560
 tgcataggca ttaactagat aaatagagag aaggatgaac caacattgca ggtagaggga 1620
 acagaatatg caaaggcagg aaggattatg gagtcgttgg aggacctgaa taaaggccca 1680

THIS PAGE BLANK (USPTO)

gtgtaagtgg	atctcagaaa	acaggaggaa	aggtgtatga	gatgagatca	gagaggcaga	1740
tcatgtgggg	tatggttaat	gttttggact	tttctattaa	gagcaatggg	gagacagtga	1800
caggacttaa	acggggaaat	aatatgacca	gattaaactt	tctaaaaaac	cctctatgca	1860
aatatatatt	gagagttaat	tattgacaaa	gattcaaagg	caacaaagtg	gagagagaat	1920
agtattttca	aaaaatgggtg	ccaaaacaat	aggacatcta	tattaaaagt	tgggtatctg	1980
tctacaaaaac	ttaattcaaaa	atggatcaca	gacctaaatg	taaaactgaa	agctatacaa	2040
cttctggaag	gaaaacacag	atgggaatct	gtgtgatctt	gagtttgaaa	atgatttatt	2100
atatctgaca	ccataatccg	taagttaaca	taattcataa	gtgaacaaag	tgatgaactg	2160
gacttcatca	gaatttaaaa	tgtttgtgct	tcaaaagaca	ctggtatgat	aatgaagaca	2220
aactacagat	aagatattgt	tgaatcatat	ttctgataaa	ggaattgtgg	ctcagaatac	2280
ataactctaa	acccccataa	taaattacaa	gtagcccaat	taaaaaaaaa	aaaagagaaa	2340
aaattttacag	tcttcatcaa	agaaagtatc	aattgtaaaa	taagcacatg	aaaaatgctc	2400
tgcatcttta	ttcatggggg	gatgaaataa	aaattaaatg	ggaaagacac	ctctaattag	2460
aatactaaaa	ttaaaaagac	tgaccatacc	aagtattggt	gaagtggaaa	tgtaaaatga	2520
tacaatcaac	ttaggtagat	gatttgggaag	tttcttacaa	aagtaggtgt	atacctaccc	2580
tgtgactcac	ccattccatg	gctaagtatt	tacctgagag	aaatgaaaga	atacatccat	2640
acaaagatgt	ttatacaaat	atttatagca	gttttatttg	tagtagcccc	aaactgaaaa	2700
gaacccaaat	gtccatcaaa	agtgaatgga	taaacaaagc	gtggtacagc	aatgcaatag	2760
aatactactt	agcaataaag	aagaatgagc	tagtgatata	cataacagct	taaatgtaca	2820
tcaaaggcat	tgtgctcagt	gaaagatgca	agtaaaaaaa	aaaaagagta	catgctgtat	2880
agttccattg	acataaaact	ctggaaagtg	aaaaacagtc	tatactgaca	gaaagcagat	2940
cattgggttg	ctgaggagga	ggagtatagg	agaggtggag	ggaaaatgta	caaagtggca	3000
caataaaaaac	ttttggaatc	atagatatat	tcactatctt	gattgagtga	tgatttcatg	3060
agtgcacgtg	cgtgtgtcaa	aatgatcaa	tttatgcaac	tttaaatatg	tgcagtttat	3120
tgtatatatc	aattatacct	cagtacggct	attaaaaaga	aacctctctg	ctgcacaatg	3180
cagaactgat	tctaggaaag	agtggaggga	ggatgaccat	ttacagtgtc	ccagggtggaa	3240
gagaacgggtg	ccttctggaa	gtgaactagg	ttggcaacaa	cagagatgaa	ataaatgggc	3300
agatgtgtga	gatacttagg	aaataaaacc	cgatggtcac	cattttccaa	aggtcagctc	3360
atcctggctt	tccagagcaa	agagctaggg	aagactttat	taataaatcc	ctcttgaagt	3420
tgcagaggaa	gcttatagca	gaaacttact	ctcaacctga	ctaactctgag	agaacacctc	3480
tggttccatt	tgattactaa	aaaactgcaa	agaacaggag	gagaaagaag	aagaaagctg	3540
gtacaaacag	tgaacttata	taatattaat	caataattgt	ctcttgttct	taaaagcaat	3600
gggaagaaaa	tgagatttga	gctggaagat	cagagttcaa	aatccaaata	aagtatatgg	3660
ccctaatatg	cttatagtag	ttaacctttc	ctgataatga	tataattgtt	gacagcacca	3720
tctttaaaat	aaaataacat	agtaatcctt	cagatttgta	gaagatcttt	cctgtttaca	3780
agtttgttct	atacacatta	tgtcttttaa	atgacacact	agccttctga	gggtaactta	3840
tattggcaac	agttttcaga	tgtggaaact	gtgaagacaa	tgttggatga	gtggaagcaa	3900
cataaacttt	ggagtctttc	agaccaggt	ttgaatgtca	gactgctttt	tattcagagt	3960
aacttcagag	cattatttct	caccttaatt	ttttttcagg	cctctttgtg	tctatgtgtc	4020
ctcttcactc	ctgtccattg	tttcttcagt	gatttttgcc	accttccttc	actgttagtg	4080
tgtagacaca	tagttctcct	ggctctgaga	gcctatgtta	attccattct	accatcctgc	4140
cacggcccac	tcaattccta	ttgagcaatg	ctagttagaa	gttgtggtgg	gattaaatgt	4200
tgcaatgagt	attcaaatga	ggttgaagta	tctacgcatt	ctacttacat	atggtgaggt	4260
atattcaagg	aagctgtagc	cattaaaatc	tcaggaaata	atttttcacc	tcctcaggtg	4320
aaagggtctt	caggcctttg	tgttctggaa	ggttcattta	tagccatttc	ccaaatgaca	4380
atgcgattga	tgagtctaga	gtctagctca	aatagcaatg	gactggaaga	ctagttagg	4440
ttttactaat	gtggaacata	gaacaaatta	tgtccttggt	tcagcctggt	catctgtgaa	4500
atagagccta	tcatatccag	tcttccctgc	cttttaggtt	gagttacctt	ctttgggtcaa	4560
ggtaagttaa	tgccatgat	gtttggctgt	gcacaagata	aagctacaac	aaagctacaa	4620
cccatctttt	ctctgtagaa	gactcaaaaa	gcaaaagaga	cccaggaaaa	tctcggaatg	4680
acttttgga	cagagagcct	ccccagaatc	agaagtcaag	gaatttaaac	atagggaagg	4740
cccaggtctc	tactgacata	aaggaaagat	gttttcttat	aggtttcacg	tttacatttt	4800
ctctctcttg	atcccatctc	cacttgcata	tgccaccttt	acacagggtc	tatgggacct	4860
cctccacaaa	agagcagttg	cagtaacca	catcatcctc	tacgccctgg	ctgtccatca	4920
agaggcgaaa	agcagcccta	tataggttct	atccttggat	agttccagtt	gtaaagttta	4980
aaatatgcga	aggcaacttg	gaaaagcaag	cggctgcata	caaagcaaac	gtttacagag	5040
ctctggacaa	aattgagcgc	ctatgtgtac	atggcaagtg	tttttagtgt	ttgtgtgttt	5100
acctgcttgt	ctgggtgatt	ttgcctttga	gagtctggag	agtagaagta	ctggttaaag	5160
gaacttccag	acaggaagaa	ggcagagaag	agggtagaaa	tgactctgat	tcttggggct	5220
gagggttcc	agagcaaatg	gcacaatgcc	acgaggcccg	atctatccct	atgacggaat	5280
ctaaggtttc	agcaagtatc	tgctggcttg	gtcatggctt	gtcctcagt	ttgtaggaga	5340
ctctcccact	ctcccactctg	cgcgctctta	tcagtcctga	aaagaacccc	tggcagccag	5400

THIS PAGE BLANK (USPTO)

gagcaggtat	tcctatcgctc	cttttcctcc	ctccctcgcc	ccaccctggt	ggtttttttag	5460
attgggcttt	ggaaccaa	ttcctgagt	ctggcctcca	ggaaatctgg	agccctggcg	5520
cctaaacctt	ggtttaggaa	accaggagct	attcaggaag	caggggtcct	ccagggctag	5580
agctagcctc	tcctgccctc	gccacgctg	cgccagcact	tgtttctcca	aagccactag	5640
gcaggcggtta	gcgcgcggtg	aggggagggg	agaaaaggaa	aggggagggg	agggaaaagg	5700
aggtgggaag	gcaaggaggc	cggcccgggtg	ggggcgggac	ccgactcgca	aactgttgca	5760
tttgctctcc	acctcccagc	gccccctccg	agatcccggg	gagccagctt	gctgggagag	5820
cgggacggtc	cggagcaagc	ccacaggcag	aggaggcgac	agagggaaaa	agggccgagc	5880
tagccgctcc	agtgtgttac	aggagccgaa	gggacgcacc	acgccagccc	cagcccggct	5940
ccagcgacag	ccaacgcctc	ttgcagcgcg	gcggcttcga	agccgcccgc	cggagctgcc	6000
ctttcctctt	cgggtgaagt	tttaaaagct	gctaaagact	cggaggaagc	aaggaaagt	6060
cctggtagga	ctgacggctg	cctttgtcct	cctcctctcc	accccgccctc	ccccaccct	6120
gccttcccc	cctcccccg	cttctctccc	gcagctgctt	cagtcggcta	ctctcagcca	6180
acccccctca	ccacccttct	ccccaccgc	cccccgccc	ccgtcgccca	gcgctgccag	6240
cccaggtttg	cagagaggta	actccctttg	gctgcgagcg	ggcgagctag	ctgcacattg	6300
caaagaaggc	tcttaggagc	caggcgactg	gggagcggct	tcagcactgc	agccacgacc	6360
cgcctgggtta	ggctgcacgc	ggagagaacc	ctctgttttc	ccccactctc	tctccacctc	6420
ctcctgcctt	ccccaccccg	agtgcggagc	cagagatcaa	aagatgaaaa	ggcagtcagg	6480
tcttcagtag	ccaaaaaaca	aaacaaacaa	aaacaaaaaa	caagaaataa	aagaaaaaga	6540
taataactca	gttcttattt	gcacctactt	cagtggacac	tgaatttgga	aggtggagga	6600
ttttgttttt	ttcttttaag	atctgggcat	cttttgaatc	tacccttcaa	gtattaagag	6660
acagactgtg	agcctagcag	ggcagatctt	gtccaccgtg	tgtcttcttc	tgcacgagac	6720
tttgaggctg	tcagagcgct	ttttgcgtgg	ttgtctccgc	aagtttccct	ctctggagct	6780
tcccgcaggt	gggcagctag	ctgcagcgac	taccgcatca	tcacagcctg	ttgaactctt	6840
ctgagcaaga	gaaggggagg	cggggtaagg	gaagtaggtg	gaagattcag	ccaagctcaa	6900
ggatg						6905

<210> 36

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 36

cacgcgtgggt acctctagaa aataattccc aatattgaat ccc

43

<210> 37

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 37

agctggctcc ccgggatctc ggaggggcgc

30

<210> 38

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 38

cacgcgtgggt accagacagt gacaggactt aaacggggaa at

42

<210> 39

<211> 16

THIS PAGE BLANK (USPTO)

<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 39
agctggctcc ccggga

16

<210> 40
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 40
cacgcgtggt acctatacac attatgtctt ttaaatac

39

<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 41
agctggctcc ccgggatctc ggaggggcgc

30

<210> 42
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 42
cacgcgtggt acctatacac attatgtctt ttaaatac

39

<210> 43
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 43
ccgccatggt gagcttggct gaatcttcca

30

<210> 44
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 44

THIS PAGE BLANK (USPTO)

ccgggtacct gccctcgccc acgctgcgcc

30

<210> 45

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 45

agctggctcc ccgggatctc ggaggggccc

30

<210> 46

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 46

ccgggtacct gccctcgccc acgctgcgcc

30

<210> 47

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 47

agctggctcc ccgggatctc ggaggggccc

30

<210> 48

<211> 54

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 48

cagaacattt ctctatcgat aggtaccgag caggtattcc tatcgctcctt ttcc

54

<210> 49

<211> 54

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 49

ggaaaaggac gataggaata cctgctcggt acctatcgat agagaaatgt tctg

54

<210> 50

<211> 54

<212> DNA

<213> Artificial Sequence

THIS PAGE BLANK (USPTO)

<220>

<223> primer

<400> 50

cagaacattt ctctatcgat aggtaccaa tctggagccc tggcgccctaa acct

54

<210> 51

<211> 54

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 51

aggtttaggc gccagggtc cagatttggt acctatcgat agagaaatgt tctg

54

<210> 52

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 52

cagaacattt ctctatcgat aggtaccggc gttagcgcg ggtgagggga g

51

<210> 53

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 53

tctccctcac cgcgcgctaa cgccggtacc tatcgataga gaaatgttct g

51

<210> 54

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 54

cagaacattt ctctatcgat aggtaccggg aaaaggaggt gggaaggcaa ggaggcc

57

<210> 55

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 55

ggcctccttg ccttcccacc tccttttccc ggtacctatc gatagagaaa tgttctg

57

<210> 56

THIS PAGE BLANK (USPTO)

<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 56
cagaacattt ctctatcgat aggtaccctc gcaaactgtt gcatttgctc tccacctccc 60

<210> 57
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 57
gggaggtgga gagcaaatgc aacagtttgc gagggtagct atcgatagag aaatgttctg 60

<210> 58
<211> 65
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 58
ccagtgtgt acaggagccg aagggaagca ccccatggaa gacgccaaaa acataaagaa 60
aggcc 65

<210> 59
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 59
cctttcttta tgtttttggc gtcttccatg ggggtgcgtcc ctccggctcc tgtacagcac 60
tgg 63

<210> 60
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 60
ccacaggcag aggaggcgac agagggccat ggaagacgcc aaaaacataa agaaaggcc 59

<210> 61
<211> 57
<212> DNA
<213> Artificial Sequence

<220>

THIS PAGE BLANK (USPTO)

<223> primer

<400> 61

cctttcttta tgtttttggc gtcttccatg gccctctgtc gcctcctctg cctgtgg 57

<210> 62

<211> 63

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 62

gggagagcgg gacgggtccgg agcaagccca ccatggaaga cgccaaaaac ataaagaaag 60
gcc 63

<210> 63

<211> 63

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 63

ggcctttctt tatgtttttg gcgtcttcca tgggtgggctt gctccggacc gtcccgtctt 60
ccc 63

<210> 64

<211> 17

<212> DNA

<213> Homo sapiens

<400> 64

tgttgcattt gctctcc 17

<210> 65

<211> 16

<212> DNA

<213> Homo sapiens

<400> 65

gctctccacc tcccag 16

<210> 66

<211> 25

<212> DNA

<213> Homo sapiens

<400> 66

ggtgagggga ggggagaaaa ggaaa 25

<210> 67

<211> 2167

<212> DNA

<213> Homo sapiens

<400> 67

caggccccac aaaacctaga tctgccccag tataactaaa tctgggacca tttattgagc 60
aattattatg tgccaagtat tgcgctgagt gcttccagag cattatctcc ttttaaccca 120
gcatagtatg tcagatgctg ttttacagat gagccaactg agaccagaga tgctcagtca 180

THIS PAGE BLANK (USPTO)

cttgcccaag	gtgacatgac	tgatatggaa	tagagtcaag	atTTTTTTTT	TTTTTTTTga	240
cacggagtct	cactctgtct	cccaggctgg	agtgcagagg	cgcaatctca	gctcactgca	300
agctctgcct	cccagggttca	cgcattctcc	tgccctcagcc	tcctgagtag	ctgggactac	360
aggcaccgcg	caccacacct	ggctaatttt	ttgtattttt	agcagagaca	gggtttcacc	420
gtgttagcca	ggatgggtctc	gatctcctga	cctcgtgatc	tgccctgcctc	ggcctcccaa	480
agtgatggaa	ttacagggtgt	gagccaccgc	gactggccag	attcaagatt	tgaaccagg	540
tcctcttggg	cccagaggcc	cctgtttctc	aactccctag	catgcatacg	cacctgtccc	600
tctagagggtg	cctgcttaag	tgtgctcagc	acatggaagc	aagttagaaa	tgctaggtat	660
acctgtaaaag	aggtgtggga	gatggggggg	agggagagaga	gaaagagatg	ctgggtgtcct	720
tcattctcca	gtccctgata	gggtgcctttg	atcccttctt	gaccagtata	gctgcattct	780
tggtctggggc	attccaacta	gaactgccaa	atthagcaca	taaaaataag	gaggcccagt	840
taaatttgaa	tttcagataa	acaatgaata	atgtgttagt	ataaatatgt	cccatgcaat	900
atcttgttga	aattaaaaaa	aaaaaaaaaa	gtcttccttc	catccccacc	cctaccacta	960
ggcctaagga	atagggtcag	gggtccaaa	tagaatgtgg	ttgagaagtg	gaattaagca	1020
ggctaataga	aggcaagggg	caaagaagaa	accttgaatg	cattgggtgc	tggtgtcctc	1080
cttaaataag	caagaagggt	gcattttgaa	gaattgagat	agaagtcttt	ttgggtctgg	1140
tgcatgttgc	cgtggttgta	attccagcac	tttgggaggc	tgaggcggga	ggatcacctg	1200
agcttgggag	ttcaagacca	gcctcaccaa	cgtggagaaa	ccctgtcttt	actaaaaata	1260
caaaaaattc	agctgggtcat	ggtggcacat	gcctgtaatc	ccagctgctc	gggaggctga	1320
ggcaggagaa	tcacttgaac	cagggaggca	gaggttgtgg	tgagcagaga	tcgcgccatt	1380
gctctccagc	ctgggcaaca	agagcaaaaag	ttcgtttaaa	aaaaaaaaaa	agtcctttcg	1440
atgtgactgt	ctcctcccaa	atgtgttagac	cctcttaaga	tcattgtttt	cagatacttc	1500
aaagattcca	gaagatatgc	cccgggggtc	ctggaagcca	caaggtaaac	acaacacatc	1560
cccctccttg	actatcaatt	ttactagagg	atgtggtggg	aaaaccatta	tttgatatta	1620
aaacaatagg	cttgggatgg	agtaggatgc	aagctcccca	ggaagttaga	taactgagac	1680
ttaaagggtg	ttaagagtgg	cagcctaggg	aaatttatcc	cggactccgg	gggagggggc	1740
agagtcacca	gcctctgcat	ttagggatcc	tccgaggaaa	agtgtgagaa	cggctgcagg	1800
caaccagggc	gtcccggcgc	taggaggggc	gacccaggcc	tgcgcgagaa	gagggagaaa	1860
gtgaagctgg	gagttgccga	ctcccagact	tcgttggaat	gcagttggag	ggggcgagct	1920
gggagcgcg	ttgctcccaa	tcaccggaga	aggaggaggt	ggaggaggag	ggctgcttga	1980
ggaagtataa	gaatgaagtt	gtgaagctga	gattcccctc	cattgggacc	ggagaaacca	2040
ggggagcccc	ccgggcagcc	gcgcgcccct	tcccacgggg	ccctttactg	cgccgcgcgc	2100
ccggccccca	cccctcgcag	cacccgcgcg	cccgcgcctt	cccagccggg	tccagccgga	2160
gccatgg						2167

<210> 68

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 68

gcacgcgtaa gcttcaggcc ccacaaaacc ta

32

<210> 69

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 69

cgctcgagcc atggctccgg ctggacccgg ctggg

35

<210> 70

<211> 10

<212> DNA

<213> Homo sapiens

THIS PAGE BLANK (USPTO)

<400> 70
gaatgaagtt 10

<210> 71
<211> 15
<212> DNA
<213> Homo sapiens

<400> 71
cgcttgctcc caatc 15

<210> 72
<211> 13
<212> DNA
<213> Homo sapiens

<400> 72
gaggaaggta taa 13

<210> 73
<211> 135
<212> DNA
<213> Escherichia coli

<400> 73
gacgtcaggt ggcacttttc ggggaaatgt gcgcggaacc cctatttggt tatttttcta 60
atacattcaa atatgtatcc gctcatgaga caataaccct gataaatgct tcaataatat 120
tgaaaaagga agagt 135

<210> 74
<211> 136
<212> DNA
<213> Escherichia coli

<400> 74
gacgtcaggt ggcacttttc ggggaaatgt gcgcggaacc cctatctggt tgttcttcta 60
gacacattca cacatgtatc cgctcatgag acaataaccc tgataaatgc ttcaatgaca 120
ttgagaaagg aagagt 136

<210> 75
<211> 12
<212> DNA
<213> Escherichia coli

<400> 75
aatacattca aa 12

<210> 76
<211> 12
<212> DNA
<213> Escherichia coli

<400> 76
catgagacaa ta 12

<210> 77
<211> 12
<212> DNA
<213> Escherichia coli

<400> 77
accctgataa at 12

THIS PAGE BLANK (USPTO)

<210> 78
<211> 12
<212> DNA
<213> Escherichia coli

<400> 78
ttgaaaaagg aa

12

THIS PAGE BLANK (USPTO)